

**BIOMIMETIC SYNTHESIS AND STRUCTURAL  
REVISION OF THE BUTANOLIDE MICROBIAL  
AUTOREGULATORS**

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the Degree of

Doctor of Philosophy

of

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Research School of Chemistry

by

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I would like to express my gratitude to all who assisted me during this journey. While it is not possible to name everyone, some deserve special thanks.

My supervisor, Professor Paul Richards, for initiating this project, for helpful suggestions and constant encouragement and for showing me the value of a healthy skepticism for the sciences and of a good sharp pencil.

## DECLARATION

This thesis contains no material previously submitted for a degree in any other university, and to the best of my knowledge and belief contains no material published or written by another person, except where due reference is made in the text.



Roger Waring (May, 1994)



## ACKNOWLEDGMENTS

*I would like to express my gratitude to all who assisted me during this project. While it is not possible to name everyone, some deserve special thanks:*

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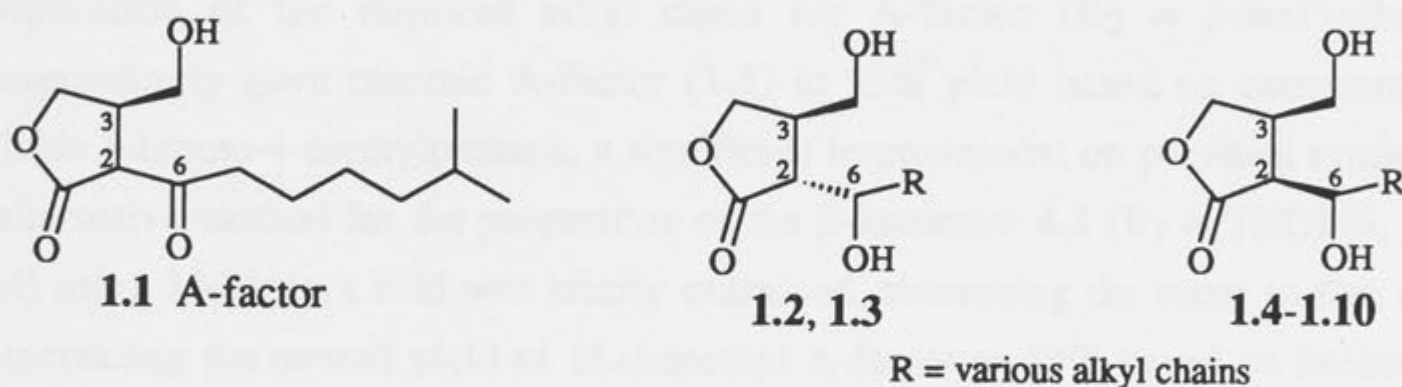
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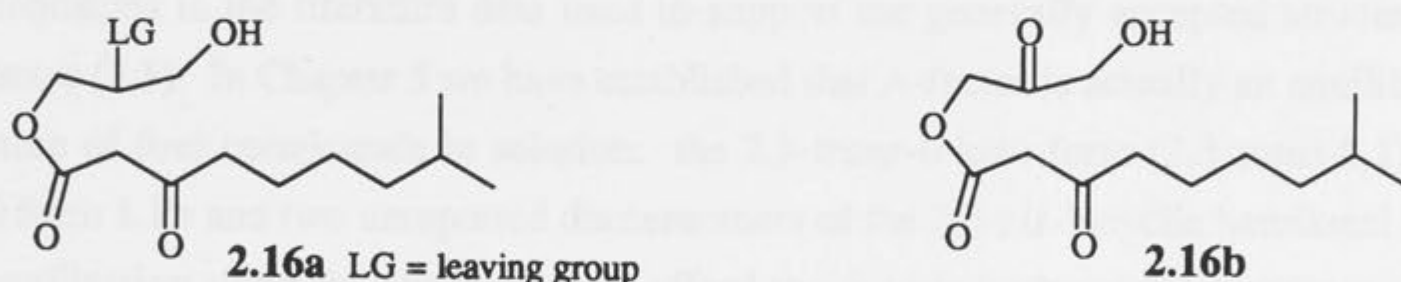


## ABSTRACT

An intriguing feature of the *Actinomycete* bacteria which produce most of the known antibiotics is their use of secondary metabolites to control aspects of their growth and metabolism. The most studied of these 'autoregulators' is the expanding group of related butanolides, previously attributed the structures **1.1-1.10**, which are potent inducers of antibiotic production and/or morphological changes in various *Streptomyces* species. A-factor (**1.1**) was the first discovered and is one of the most active of these metabolites. The work described in this thesis centres on the development of an improved synthetic route to these metabolites based on our proposal for their biosynthesis.



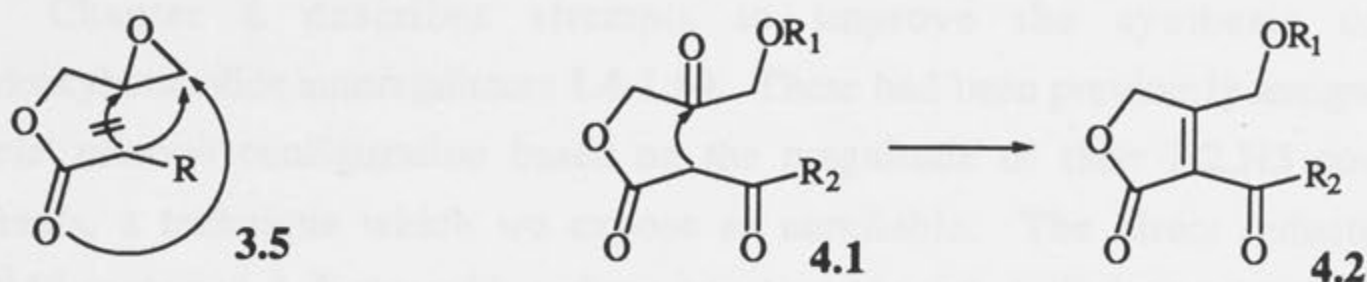
In Chapter 2 we propose a biosynthetic origin of A-factor (**1.1**) in which the key step is the creation of the C2-C3 butanolide bond by the cyclisation of a  $\beta$ -ketoester of a 1,3-diol. Two plausible methods of cyclisation are proposed: intramolecular alkylation *via* **2.16a** or Knoevenagel-style cyclisation *via* **2.16b**.



Chapter 3 describes attempts to model the former process by base catalysed cyclisation of glycidyl esters **3.5**, a seemingly very direct route to the required 3-hydroxymethylbutanolides. In fact none of the required C-alkylation at the secondary epoxide site was observed with either simple esters (**3.5**, R = methyl or phenyl) or with a  $\beta$ -ketoester (R = heptanoyl). The only intramolecular reaction products observed resulted from either C-alkylation at the alternative primary epoxide site or O-alkylation. Even with a primary acyclic leaving group (*i.e.* the 2-iodoethyl ester), exclusive O-alkylation was still observed. Analysis of the reaction geometry reveals that severe stereoelectronic constraints disfavour the required cyclisation.

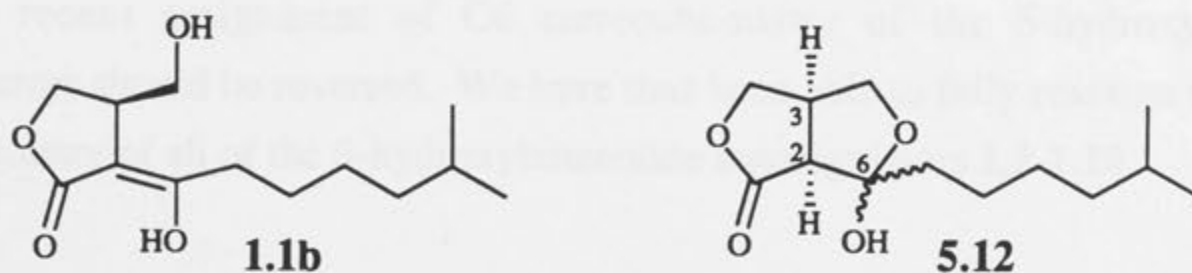


ABSTRACT (CONTINUED)



By contrast, the Knoevenagel cyclisation of a  $\beta$ -ketoester of dihydroxyacetone (**4.1**,  $R_1$  = TBDMS,  $R_2$  = hexyl) examined in Chapter 4 gave in good yield the corresponding butenolide **4.2**, which upon further elaboration afforded 11-demethyl A-factor in six steps from ethyl acetoacetate with an overall yield of 20%. Incorporation of the required alkyl chain for A-factor ( $R_2$  = 5-methylhexyl) correspondingly gave racemic A-factor (**1.1**) in 33% yield based on commercially available 1-bromo-4-methylpentane, a significant improvement on previous syntheses. An alternative method for the preparation of the  $\beta$ -ketoester **4.1** ( $R_1$  = TBDMS,  $R_2$  = hexyl) using Meldrum's acid was briefly examined, shortening the route to five steps and increasing the overall yield of 11-demethyl A-factor to 48% based on Meldrum's acid. The efficiency of the synthetic route provides support for the proposal that a similar cyclisation occurs in the biosyntheses of the butanolide autoregulators (**1.1-1.10**).

Preparation of synthetic A-factor brought to our attention the surprising inadequacies in the literature data used to support the generally accepted structure of A-factor (**1.1**). In Chapter 5 we have established that A-factor is actually an equilibrium mixture of four compounds in solution: the 2,3-*trans*-6-keto form (2,3-*trans*-**1.1**), the enol form **1.1b** and two unreported diastereomers of the 2,3-*cis*-bicyclic hemiketal **5.12**. Crystallisation shifts the equilibrium to afford the 6-*endo*-hydroxy diastereomer of the hemiketal **5.12**, the configuration of which has been defined by X-ray diffraction. A-factor has been shown to readily form ketals with alcohols in the presence of an acid catalyst.





## ABSTRACT (CONTINUED)

Chapter 6 describes attempts to improve the synthesis of the 6-hydroxybutanolide autoregulators **1.4-1.10**. These had been previously assigned the '2,3-*cis*' relative configuration based on the magnitude of their H<sub>2</sub>,H<sub>3</sub> coupling constants, a technique which we expose as unreliable. The direct reduction of TBDMS-protected A-factor with sodium borohydride and desilylation gave racemic factor-I (**1.2**, R = 5-methylhexyl, 65% yield) and Gräfe's factor (**1.5**, R = 5-methylhexyl, 25% yield), respectively. In order to improve the yield of the more active, putatively '2,3-*cis*' diastereomer we developed a novel approach to the diastereospecific formation of 2,3-*cis* butanolides *via* the catalytic hydrogenation of the protected 6-hydroxybutenolide **6.15**. This reaction successfully produced two 2,3-*cis*-6-hydroxybutanolide derivatives, but surprisingly *neither* of them corresponded the *O*-TBDMS derivative of autoregulator **1.5**. NOESY spectroscopy confirmed that the hydrogenation products **6.16** had 2,3-*cis* relative geometry and that the *O*-TBDMS derivatives of *both* of the racemic natural compounds (±)-**1.2** and (±)-**1.5** had 2,3-*trans* relative stereochemistry, contrary to literature reports. By analogy, all of the former '2,3-*cis*' 6-hydroxybutanolide autoregulators (**1.4-1.10**) must be reassigned as 2,3-*trans* and thus the two classes of 6-hydroxybutanolide autoregulators **1.2**, **1.3** and **1.4-1.10** differ only in their relative configuration at C<sub>6</sub>.

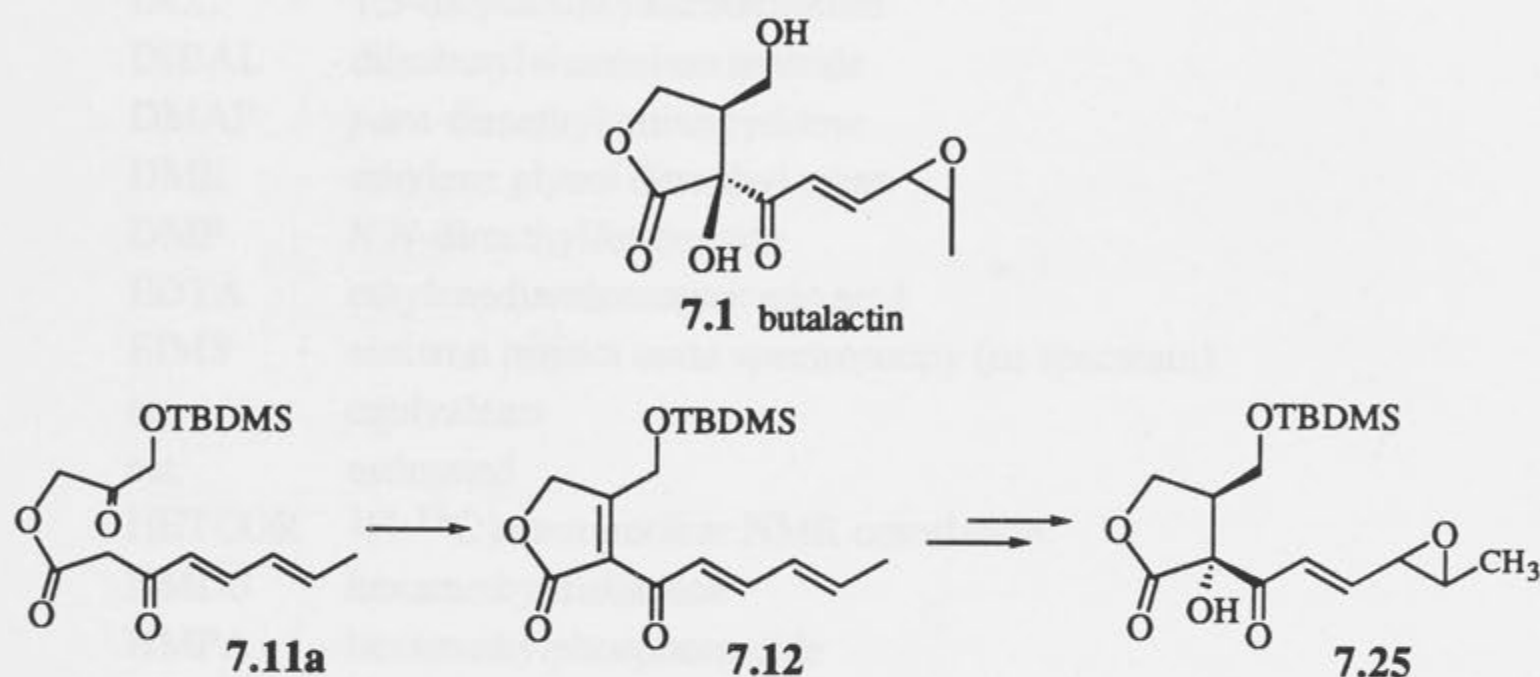


We have further demonstrated that the recently reported absolute stereochemical assignment of the C<sub>6</sub> hydroxyl group of the natural 6-hydroxybutanolide autoregulators is also likely to be erroneous. Detailed analysis of the stereochemical outcome of our sodium borohydride reduction of TBDMS-protected A-factor supports our prediction that this recent assignment of C<sub>6</sub> stereochemistry of the 6-hydroxybutanolide autoregulators should be reversed. We have thus been able to fully reassign the relative stereochemistry of all of the 6-hydroxybutanolide autoregulators **1.2-1.10**.



# ABSTRACT (CONTINUED)

In the final chapter, a model biomimetic synthesis of the non-autoregulatory butanolide metabolite, butalactin (7.1), has been developed as an extension of our synthesis of A-factor. Knoevenagel cyclisation of the dienyl  $\beta$ -ketoester 7.11a gave the butenolide 7.12 which was elaborated to a diastereomeric mixture of epoxides 7.25. These are protected stereoisomers of butalactin 7.1, demonstrating that all of the functionality required for the first total synthesis of butalactin (7.1) can be installed.



## ABBREVIATIONS

AIBN	2,2'-azobisisobutyronitrile
APT	attached proton test
BOM	benzyloxymethyl
cat.	catalyst
CIMS	chemical ionisation mass spectroscopy (or spectrum)
COSY	$^1\text{H}$ - $^1\text{H}$ correlation NMR spectroscopy
<i>m</i> -CPBA	<i>meta</i> -chloroperbenzoic acid
DCC	1,3-dicyclohexylcarbodiimide
DIBAL	diisobutylaluminium hydride
DMAP	<i>para</i> -dimethylaminopyridine
DME	ethylene glycol dimethyl ether
DMF	<i>N,N</i> -dimethylformamide
EDTA	ethylenediaminetetraacetic acid
EIMS	electron impact mass spectroscopy (or spectrum)
eq.	equivalents
est.	estimated
HETCOR	$^1\text{H}$ - $^{13}\text{C}$ heteronuclear NMR correlation
HMDS	hexamethyldisilazane
HMPA	hexamethylphosphoramide
HPLC	high pressure liquid chromatography
HRMS	high resolution mass spectroscopy
LiHMDS	lithium hexamethyldisilazide
MTPA	$\alpha$ -methoxy- $\alpha$ -trifluoromethylphenyl acetate
MPLC	medium pressure liquid chromatography
MW	molecular weight
NADPH	nicotinamide-adenine dinucleotide phosphate
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser effect spectroscopy
py.	pyridine
$R_f$	retardation factor
<i>S.</i>	<i>Streptomyces</i>
TBDMS	<i>tert</i> -butyldimethylsilyl
TBDMSCl	<i>tert</i> -butyldimethylsilyl chloride
THP	tetrahydropyran
TLC	thin layer chromatography
TMS	trimethylsilyl
Ts	<i>para</i> -toluenesulfonyl
TsCl	<i>para</i> -toluenesulfonyl chloride
VB-	virginiae butanolide
xs	excess



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*Nature uses as little as possible of anything*

1.6 SYNTHESIS OF THE ACTINOMYCETE AUTOREGULATOR

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Actinomycetes

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Actinomycetes and the Actinomycetozoa

Actinomycetes and the Actinomycetozoa

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Johannes Kepler

(1571-1630)

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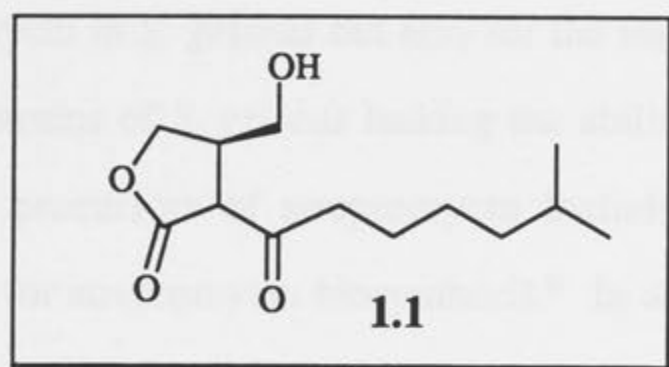
## 1.1 INTRODUCTION TO ACTINOMYCETE AUTOREGULATORS

Of the more than 6,000 antibiotics discovered to date, most have been isolated from bacteria of the order *Actinomycetales*.<sup>1</sup> Between 1971 and 1980, 75% of newly isolated antibiotics were derived from members of this important group of microbial genera, with the remainder originating largely from fungi and other classes of unicellular bacteria.<sup>2</sup>

Actinomycetes are multicellular, Gram-positive bacteria, typically isolated from soils, and characterised by their relatively complex morphological development, generally involving filamentous growth, development of aerial mycelia and spore formation. This complex morphology appears to be related to their ability to produce a wide range of secondary metabolites, including antibiotics.<sup>3</sup>

An intriguing feature of this class of microorganisms is their use of self-generated signal compounds or 'autoregulators' to control aspects of cellular differentiation and/or the production of secondary metabolites including antibiotics. These compounds, of which A-factor (1.1) is the archetypal representative, act at extremely low concentrations in a manner analogous to hormones in higher organisms, to control aspects of growth and metabolism in disassociated parts of the organism. The study of these compounds has contributed greatly to the understanding of the regulatory systems of these industrially important microorganisms, in which progress is charted through numerous reviews on the subject.<sup>3-7</sup>

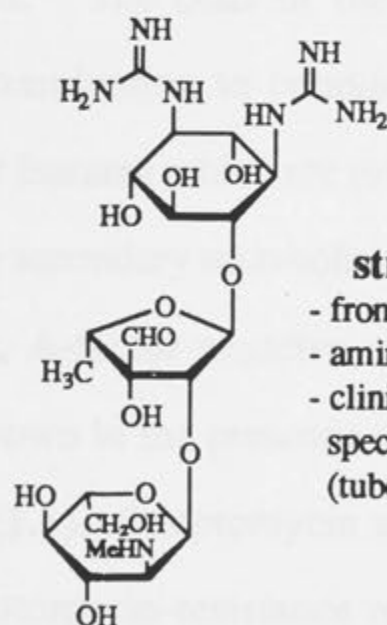
## 1.2 A-FACTOR



### A-factor

- isolated from *S. griseus*
- potent regulator of:-
  - streptomycin biosynthesis
  - streptomycin resistance
  - aerial mycelium formation and sporulation
  - formation of yellow pigment
  - leukaemomycin biosynthesis

The first discovered and most widely studied microbial autoregulator is A-factor (1.1).<sup>6</sup> It was discovered in 1967 by Khokhlov and coworkers<sup>8</sup> during investigation into the mechanism of biosynthesis of streptomycin, a clinically important broad spectrum aminoglycoside antibiotic produced by *Streptomyces griseus*. They found that addition of a small amount of the culture broth of a normal streptomycin producing strain of *S. griseus* restored the ability to produce the antibiotic to cultures of some non antibiotic-producing mutant strains of the same species. This treatment also restored the ability of these mutant strains to produce spores, and suggested the presence of a multi-functional regulatory factor in wild-type *S. griseus* strains which was absent in the mutant strains.



### streptomycin

- from *S. griseus*
- aminoglycoside antibiotic
- clinically used broad spectrum antibacterial (tuberculostatic) agent

Isolation and characterisation of the autoregulatory factor 'A-factor' proved difficult due to its extremely low concentration in the culture broth (*ca* 30 µg/L).<sup>6</sup> After it was eventually purified and characterised as the surprisingly simple γ-lactone 1.1



[2-(6-methylheptanoyl)-3-hydroxymethylbutanolide],<sup>9</sup> the full extent and potency of its biological actions were revealed.

A-factor (1.1) was found to be essential not just for the production of streptomycin in *S. griseus* but also for the initiation of its entire biosynthetic pathway. Mutant strains of *S. griseus* lacking the ability to produce A-factor are also unable to produce precursors of streptomycin including streptidin or many of the enzymes required for streptomycin biosynthesis.<sup>6</sup> In addition, A-factor (1.1) has been found to control the production of the streptomycin-inactivating enzyme, streptomycin-6-phosphotransferase (the enzyme responsible for conferring resistance to streptomycin on the producing organism<sup>10</sup>) as well as more than 10 other unidentified proteins.<sup>3</sup> A-factor was also found to induce the production of a diffusible yellow pigment which contains an amino sugar moiety.<sup>11</sup>

Combined with these effects of A-factor on secondary metabolism are its regulatory influences on aspects of cellular differentiation and morphological development. The normal morphological development of wild-type *S. griseus* on an agar medium consists of filamentous mycelial growth followed by the formation of aerial mycelia from which spore chains develop. However, A-factor deficient mutant strains simply form large, diffuse filamentous colonies which fail to develop either aerial mycelia or spores.<sup>6</sup> The cells of the mutant strains have also been seen by electron microscopic examination to contain very few intracellular membranes and other distinctive cellular features which are present in the wild strain.<sup>6</sup> Remarkably, the profound deficiencies in secondary metabolite formation and cellular differentiation that are observed in mutant, A-factor negative strains can be almost completely restored when the mutants are grown in the presence of minute quantities (*ca* 0.2 ng/mL) of the autoregulator A-factor (1.1). Streptomycin and other secondary metabolites are then produced as usual, streptomycin-resistance returns<sup>10</sup> and normal sporulation occurs.<sup>6</sup> Gräfe and coworkers have shown that A-factor also regulates the production of the anthracyclin-type antibiotic, leukaemomycin in other strains of *S. griseus*.<sup>5</sup>

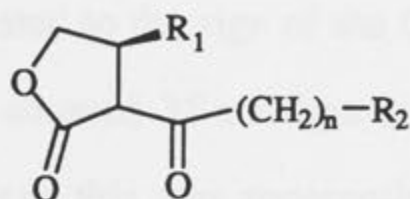


The addition of 1 ng of A-factor can induce the formation of  $1 \times 10^6$  ng (1 mg) of streptomycin; an induction coefficient of *one million*.<sup>6</sup> Such is the biological activity of this compound that it can be detected by bioassay at the picomolar ( $10^{-12}$  M) level making it one of the most potent natural bioregulators.<sup>4</sup>

Three surveys of other actinomycete species<sup>12-14</sup> have shown that substances giving an A-factor-like response in mutant indicator-strains of *S. griseus* are widely distributed in other species. In total, 23% of the 510 *Streptomyces* strains tested (> 100 species) and 15% of the 102 other actinomycete strains tested (> 40 species) showed a positive response, but the identity of most of these substances and their role in the producing organisms are yet to be determined. It is clear that A-factor-like microbial hormones are widespread among actinomycetes and considerable effort has been directed towards their identification and the understanding of their metabolic role.<sup>3</sup>

There appear to be rather strict structural requirements for autoregulatory activity in *S. griseus*, since Khokhlov reports that almost any alteration to the structure of A-factor (1.1) causes a sharp drop or complete loss of activity as shown in Table 1.1.<sup>4</sup> Activity of the O-acyl derivatives (Entries 10, 12) was attributed to their likely enzymatic conversion to A-factor (Entry 1) *in vivo* during the lengthy biological testing procedure.<sup>15</sup>

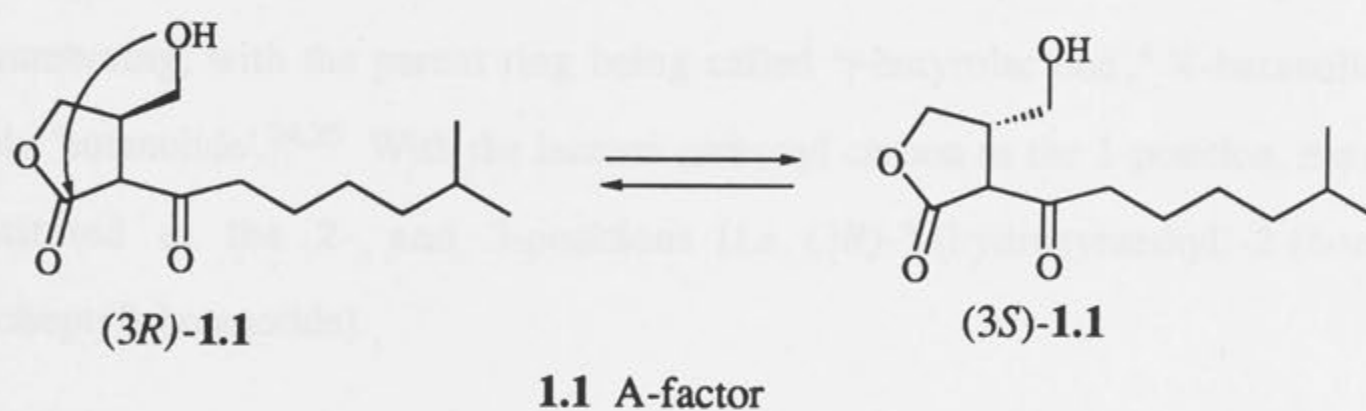
**Table 1.1 Structure-Activity Study by Khokhlov *et al.* for Autoregulator Activity in *S. griseus***



Entry	R <sub>1</sub> =		n =	R <sub>2</sub> =	Relative Activity
					(%)
1	CH <sub>2</sub> OH	(A-factor)	4	CHMe <sub>2</sub>	100
2	CH <sub>2</sub> OH		3	CHMe <sub>2</sub>	0-10
3	CH <sub>2</sub> OH		2	CHMe <sub>2</sub>	< 0.1
4	CH <sub>2</sub> OH		5	CHMe <sub>2</sub>	0-15
5	CH <sub>2</sub> OH		4	Me	< 0.1
6	CH <sub>2</sub> OH		5	Me	0-20
7	CH <sub>2</sub> OH		6	Me	0-10
8	CH <sub>2</sub> OH		7	Me	< 0.1
9	CH <sub>2</sub> OH		8	Me	< 1
10	CH <sub>2</sub> OCOCH <sub>3</sub>		4	CHMe <sub>2</sub>	~ 100
11	CH <sub>2</sub> OCOPh		4	CHMe <sub>2</sub>	0-10
12	CH <sub>2</sub> OCO(CH <sub>2</sub> ) <sub>4</sub> CHMe <sub>2</sub>		4	CHMe <sub>2</sub>	~ 100
13	CH <sub>2</sub> OCOPh		3	CHMe <sub>2</sub>	< 1
14	CH <sub>2</sub> OCOPh		2	CHMe <sub>2</sub>	< 0.1
15	CH <sub>2</sub> OCOPh		1	CHMe <sub>2</sub>	< 0.1
16	CH <sub>2</sub> OCOPh		5	CHMe <sub>2</sub>	< 0.1
17	CH <sub>2</sub> OCOPh		0	C <sub>6</sub> H <sub>5</sub>	< 0.1
18	H		4	CHMe <sub>2</sub>	< 0.1
19	CH <sub>3</sub>		4	CHMe <sub>2</sub>	< 0.1
20	CH <sub>2</sub> OTHP		4	CHMe <sub>2</sub>	< 0.1
21	OCO(CH <sub>2</sub> ) <sub>4</sub> CHMe <sub>2</sub>		0	C <sub>6</sub> H <sub>5</sub>	< 0.1



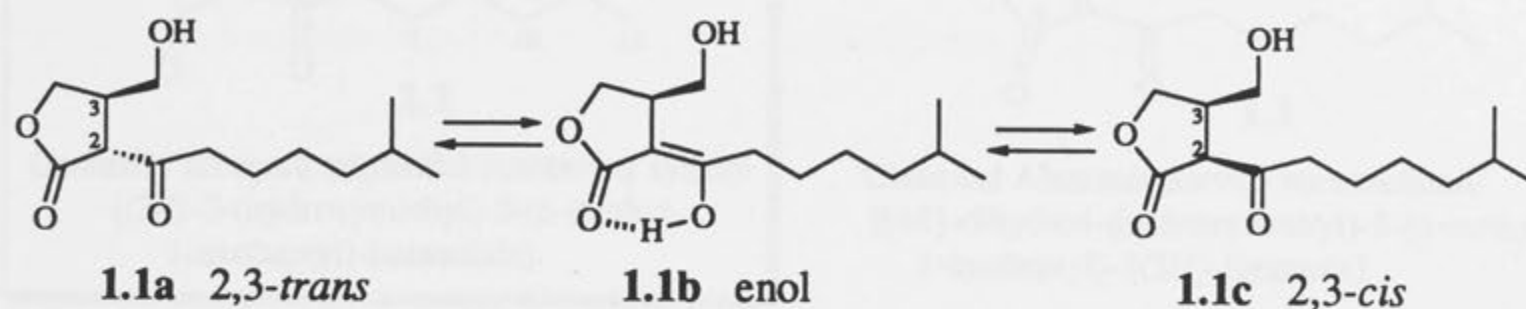
The determination of the absolute configuration of natural (-)-A-factor (**1.1**) has involved considerable confusion. Khokhlov and coworkers initially proposed a  $3R$  configuration based on Klyne's lactone sector rule,<sup>16</sup> a method in which the absolute configuration of a lactone is related to the sign of the Cotton effect in its CD spectrum, but confusingly their diagram showed  $3S$  configuration.<sup>9</sup> In later publications,<sup>6</sup> the assignment was revised to  $3S$ , and this was apparently confirmed soon after by Mori, who synthesised optically active A-factor (**1.1**) identical to the natural material, from (-)-paraconic acid (see Section 1.6.1).<sup>17</sup> Mori contributed to the confusion by referring to his nature-identical product as ' $3S$ -(+)-A-factor'<sup>17</sup> instead of  $3S$ -(-)-A-factor, an error which was corrected in a later paper in which the synthesis of both chiral forms is described.<sup>18</sup> Unfortunately, one final revision of the absolute configuration of the natural metabolite to its presently accepted designation of  $3R$ -(-)-A-factor was required when the assignment of the chiral starting material, (-)-paraconic acid, was reversed,<sup>19</sup> (see Section 1.6.1). Surprisingly, the 'unnatural'  $3S$ -enantiomer has been reported to have approximately 30-40% of the activity of 'natural'  $3R$ -A-factor,<sup>18</sup> although it has been proposed that this may be due to partial racemisation occurring *via* translactonisation during the bioassay<sup>14</sup> since this process is known to be readily catalysed by acid or base.<sup>17,20</sup>



The C2 of A-factor is likely to be readily epimerisable *via* the enol form (**1.1b**) and it has been generally assumed that the 2,3-*trans* configuration **1.1a** would predominate over the 2,3-*cis* form **1.1c** for reasons of thermodynamic stability.<sup>9,18</sup> Prior to the present work, no definitive evidence had been reported for the tautomeric contributions to the structure of A-factor (**1.1**). In Chapter 5, the assumptions



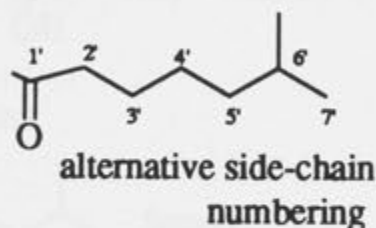
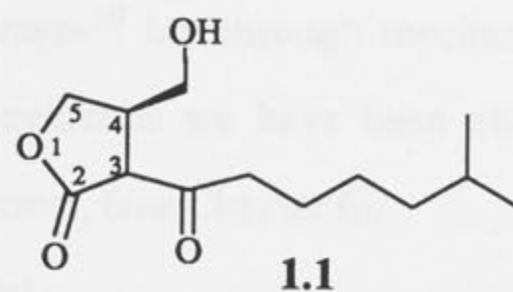
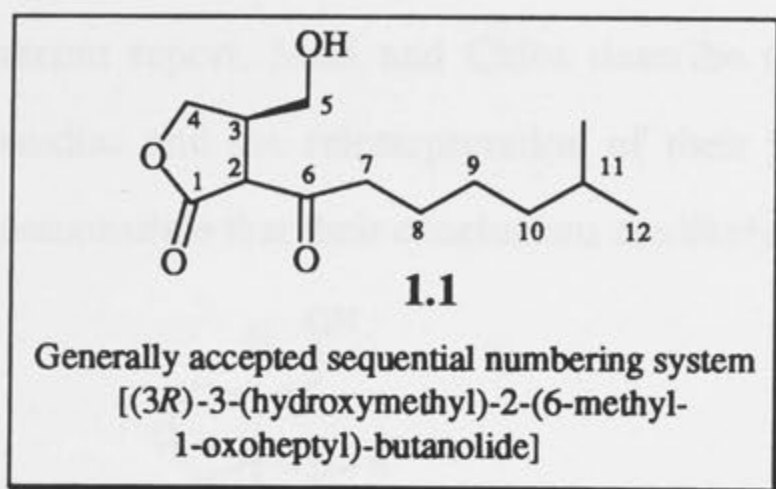
concerning the relative stereochemistry of A-factor (**1.1**) are re-examined to reveal a surprisingly complex tautomeric mixture.



Recent interest in A-factor (**1.1**) is reflected in the patent granted for its over-production by microbial genetic engineering techniques<sup>21</sup> and by its very recent appearance (in racemic form) as a widely advertised, high value commercial research reagent (A\$100 per *milligram*)<sup>22</sup> "for studying secondary metabolism and morphogenesis in Actinomycetes".<sup>23</sup>

### 1.2.1 Nomenclature and Numbering of the Butanolide Autoregulators

A-factor (**1.1**) is named in Chemical Abstracts based on dihydro-2(3*H*)-furanone [*i.e.* (4*R*)-4-(hydroxymethyl)-3-(6-methyl-1-oxoheptyl)dihydro-2(3*H*)-furanone] where the ring substituents are at the 3- and 4-positions. However, all other literature references to this and related autoregulators have used the  $\gamma$ -lactone moiety as a basis for numbering, with the parent ring being called ' $\gamma$ -butyrolactone',<sup>4</sup> '4-butanolide'<sup>5</sup> or simply 'butanolide'.<sup>24,25</sup> With the lactone carbonyl carbon as the 1-position, the ring is substituted at the 2- and 3-positions [*i.e.* (3*R*)-3-(hydroxymethyl)-2-(6-methyl-1-oxoheptyl)-butanolide].



For NMR assignments and general discussion, the carbon of the hydroxymethyl substituent is generally referred to as the 5-position and the acyl chain carbons are usually numbered sequentially starting with the carbonyl carbon as C6.<sup>3,26,27</sup> Some reports, however, use 'prime' notation for the acyl chain so that the ketone carbon is C1'.<sup>17,28</sup>

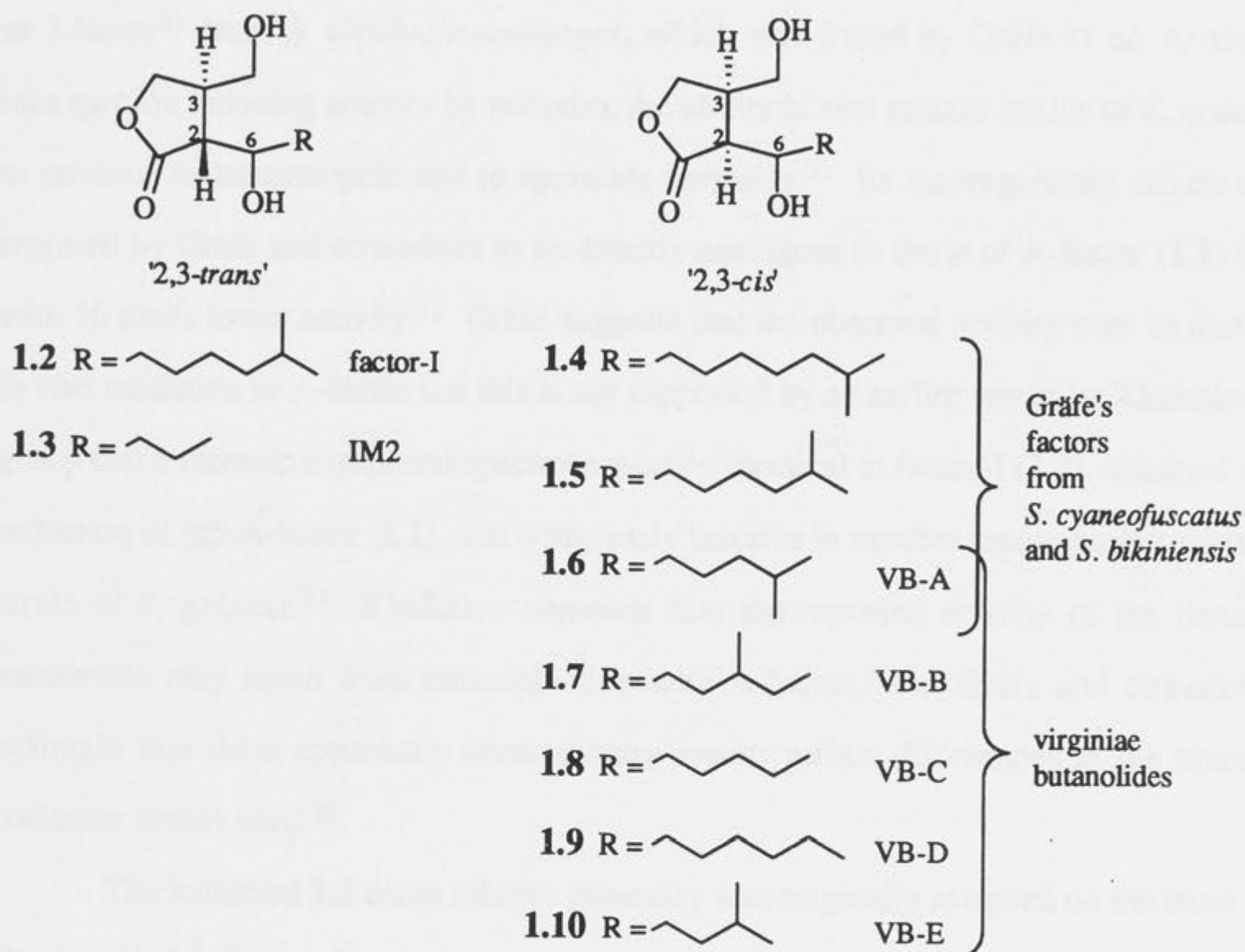
For simplicity and consistency with the majority of the more recent literature,<sup>3,29</sup> we have adopted the sequential numbering system for A-factor and related butanolides as depicted above. In this thesis, the term 'butanolide' is used to refer to the parent 2(3*H*)-dihydrofuranone.

### 1.3 REDUCED A-FACTOR HOMOLOGUES: THE 6-HYDROXYBUTANOLIDES

Following the discovery of A-factor (1.1), an expanding series of closely related regulatory compounds (1.2 -1.10) has been isolated from a variety of *Streptomyces* species, in which the 6-keto group is replaced by a 6-hydroxyl group. These compounds have been categorised as possessing either '2,3-*cis*' or '2,3-*trans*' relative stereochemistry, as shown below, but we have found evidence to undermine the basis of these assignments (see Chapter 6). At the commencement of this project the relative configuration of the 6-hydroxyl groups of these compounds had not been assigned. In a



recent report, Mori and Chiba describe this assignment<sup>30</sup> but through mechanistic studies and the reinterpretation of their published evidence we have been able to demonstrate that their conclusions are likely to be incorrect, (see Chapter 6).

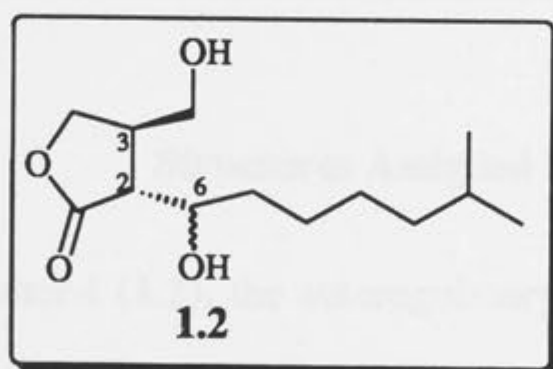


### Reported Structures of the 6-Hydroxybutanolide Autoregulators

### 1.3.1 Factor-I

The first isolated of this group was 'factor-I' (1.2)<sup>26</sup> (sometimes called 'factor 1'<sup>27</sup> or I-factor<sup>3</sup>) from *S. viridochromogenes*, which was found by Gräfe *et al.* to show interspecific inducing activity by restoring the ability of two mutant strains of *S. griseus* to produce leukaemomycin and to sporulate normally.<sup>31</sup> Its autoregulatory effects are reported by Gräfe and coworkers to be directly analogous to those of A-factor (1.1) but with 10 times lower activity.<sup>31</sup> Gräfe suggests that the observed activity may be due to *in vivo* oxidation to A-factor but this is not supported by an earlier report by Khokhlov's group that a racemic compound spectroscopically identical to factor-I (1.2), obtained *via* reduction of (±)-A-factor (1.1), was completely inactive in another asporogenous mutant strain of *S. griseus*.<sup>32</sup> Khokhlov contends that the reported activity of the natural metabolite may result from contamination with A-factor,<sup>4</sup> but Gräfe and coworkers maintain that these apparently contradictory results reflect differences in the mutant indicator strains used.<sup>26</sup>

The indicated 2,3-*trans* relative geometry was originally assigned on the basis of the  $J_{2,3}$  <sup>1</sup>H NMR coupling constant, a technique whose validity is seriously questioned in Chapter 6. The absolute configuration of factor-I (1.2) has not yet been determined.

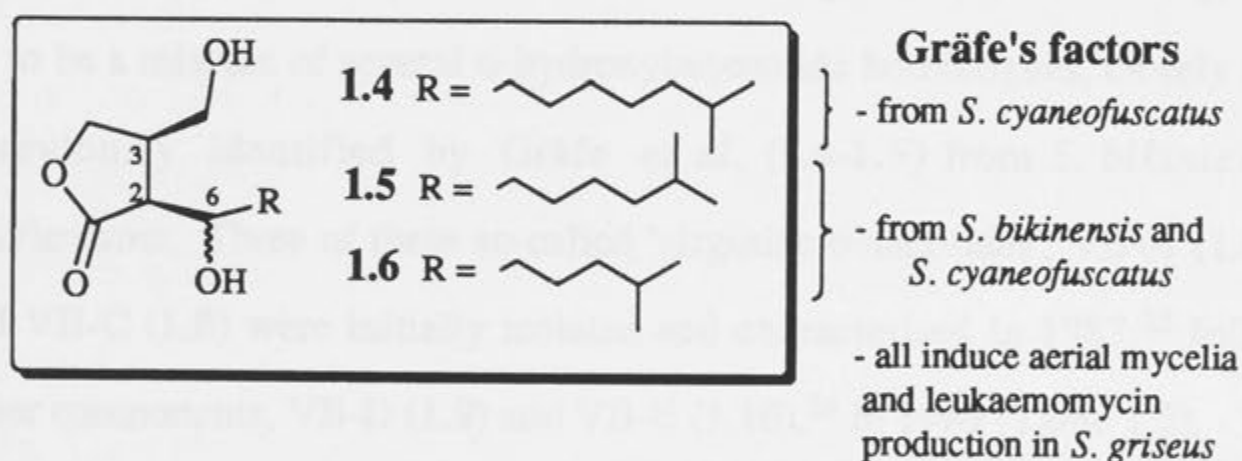
**factor-I**

-isolated from  
*S. viridochromogenes*  
-induces sporulation  
and leukaemomycin  
production in *S. griseus*



### 1.3.2 Gräfe's Factors: Interspecific Regulators from *S. bikiniensis* and *S. cyaneofuscatus*

Further work by Gräfe and coworkers resulted in the isolation of a mixture of two butanolide metabolites **1.5** and **1.6** from cultures of *S. bikiniensis* which could restore leukaemomycin production and aerial mycelium formation to blocked mutants of *S. griseus*.<sup>28</sup> The biological activity of the mixture was qualitatively and quantitatively similar to factor-I (**1.2**), with an activity approximately an order of magnitude lower than A-factor (**1.1**). The same two inducers were also isolated from cultures of *S. cyaneofuscatus*, together with a third, homologous bioregulator **1.4**. Although not separated, detailed spectroscopic analysis of the mixtures allowed the structures **1.4**, **1.5** and **1.6** to be proposed. The structures were assigned by Gräfe and coworkers to have different ('cis') 2,3-relative stereochemistry to factor-I (**1.2**) due to their lower H2-H3 <sup>1</sup>H NMR coupling constant (discussed further in Chapter 6).



#### Structures Assigned by Gräfe *et al.*

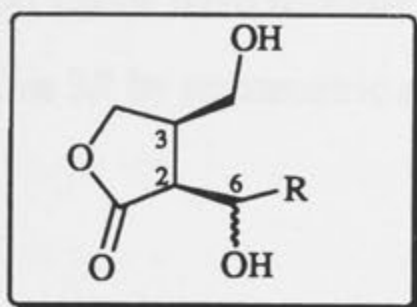
As for factor-I (**1.2**), the autoregulatory function of these interspecific inducer compounds in the producing strains remains to be demonstrated since mutants deficient in these compounds are not available for study.

### 1.3.3 The *Virginiae* Butanolides (VBs)

In 1979, Yanagimoto and coworkers reported the isolation of a partially purified autoregulatory factor for the production of the cyclic depsipeptide antibiotic, virginiamycin (staphylomycin), in cultures of *S. virginiae*.<sup>33</sup> The influence of the isolated inducing material on antibiotic biosynthesis by *S. virginiae* was found to be highly dependent on its concentration and the time of addition, with either stimulation or inhibition being observed under certain conditions.<sup>34</sup> When an adequately diluted sample was added to young cells prior to the normal onset of antibiotic production, a strong induction of virginiamycin biosynthesis could be measured, demonstrating its autoregulatory activity without the need for mutant strains. However, in contrast to A-factor (1.1), this autoregulator does not have reported effects on morphogenesis or cytodifferentiation of the producing strain.

Nearly a decade later the antibiotic inducing material in *S. virginiae* was revealed to be a mixture of several 6-hydroxybutanolide homologues, closely related to those previously identified by Gräfe *et al.* (1.4-1.6) from *S. bikiniensis* and *S. cyaneofuscatus*. Three of these so-called 'virginiae butanolides', VB-A (1.6), VB-B (1.7) and VB-C (1.8) were initially isolated and characterised in 1987,<sup>25</sup> followed by two further components, VB-D (1.9) and VB-E (1.10),<sup>24</sup> in 1989 (Table 1.2).



Table 1.2 Accepted Structures and Relative Activities of the *Virginiae* Butanolides**virginiae butanolides (VBs)**

- isolated from *S. virginiae*
- highly active inducers of virginiamycin production in *S. virginiae*

	Name	R =	Rel. Activity
1.6	VB-A		100 <sup>i</sup>
1.7	VB-B		10
1.8	VB-C		40
1.9	VB-D		60
1.10	VB-E		10

Note i) VB-A minimum effective concentration is 0.6 ng/mL

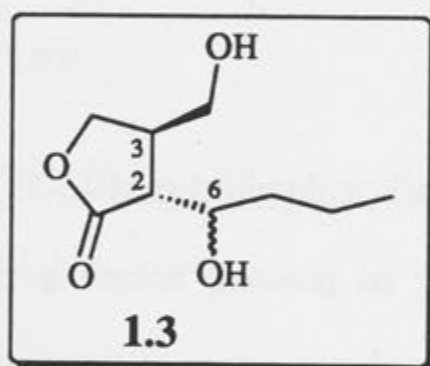
VB-A (1.6) was found to be spectroscopically identical to one of Gräfe's inducing factors (1.6, see Section 1.3.2). The *virginiae* butanolides (VBs) were all assigned '2,3-*cis*' relative stereochemistry since they shared the same  $^1\text{H}$  NMR  $J_{2,3}$  coupling value as Gräfe's factors 1.4, 1.5 and 1.6 (see Chapter 6 for criticism of this assignment).

The differences in alkyl chain length and methyl branching among the VBs result in some differences in biological activity, as shown in Table 1.2,<sup>24</sup> but the structural requirements for activity do not seem to be as rigid as for A-factor (*cf.* Table 1.1). VB-A (1.6) is the most potent VB isolated to date with a minimum effective concentration in bioassay of 0.6 ng/mL.<sup>24</sup> A structure-activity survey of numerous synthetic straight-chain VB derivatives (*cf.* VB-C, 1.8 and VB-D, 1.9) concluded that virginiamycin inducing activity was highest with a C2 hydroxyalkyl chain of 7 or 8 carbon atoms in length.<sup>35</sup> In addition, derivatives attributed '2,3-*trans*' configuration were more than 10 times less active than the corresponding '2,3-*cis*' derivatives.<sup>35</sup> Interestingly, substrates incorporating a 6-keto group (*cf.* A-factor 1.1) generally had over 100 times lower activity than the corresponding '2,3-*cis*'-6-hydroxy compounds,

demonstrating species discrimination between butanolides with 6-keto and 6-hydroxy groups. Mori and Chiba have recently defined the absolute stereochemistry of VB-A, VB-B and VB-C as 3*R* by asymmetric synthesis.<sup>30</sup>

#### 1.3.4 IM-2

The latest addition to the class of 6-hydroxybutanolide autoregulators is 'IM-2' (1.3),<sup>36</sup> a metabolite of *Streptomyces* sp. *FRI-5*. IM-2 was initially noted for its ability to induce the formation of a blue pigment,<sup>36</sup> but has recently been shown to also induce the formation of the nucleoside antibiotics, showdomycin and minimycin.<sup>37</sup>



#### IM-2

- from *Streptomyces* sp. *FRI-5*
- induces blue pigment formation
- induces showdomycin and minimycin biosynthesis

It is distinguished from the virginiae butanolides (see Table 1.2) by its considerably shorter C2-alkyl chain and by the '2,3-*trans*' relative stereochemistry assigned to it.<sup>36</sup> A study of the influence of C2-alkyl chain length in IM-2 analogues on inducing activity revealed that while similar activity was observed when the alkyl chain was one carbon longer, any further increase or any decrease in the C2 side chain length caused a reduction in activity of more than 50-fold,<sup>36</sup> indicating that *FRI-5* has more stringent structural requirements than *S. virginiae*.

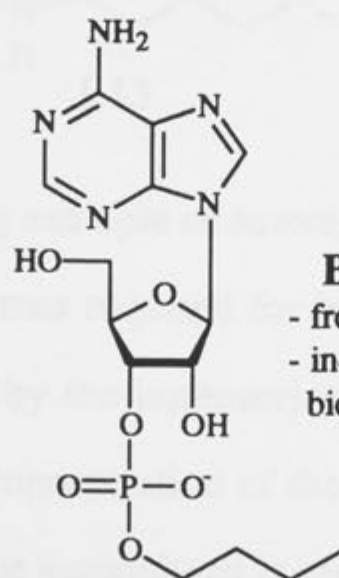


## 1.4 OTHER ACTINOMYCETE AUTOREGULATORS

In addition to the 2,3-disubstituted butyrolactone autoregulators discussed above, several other classes of actinomycete metabolites with autoregulatory functions have been reported. These will not be treated in depth here but serve to illustrate the variety of autoregulatory molecules which are utilised by actinomycetes to control aspects of their metabolism and morphology.

### 1.4.1 B-factor

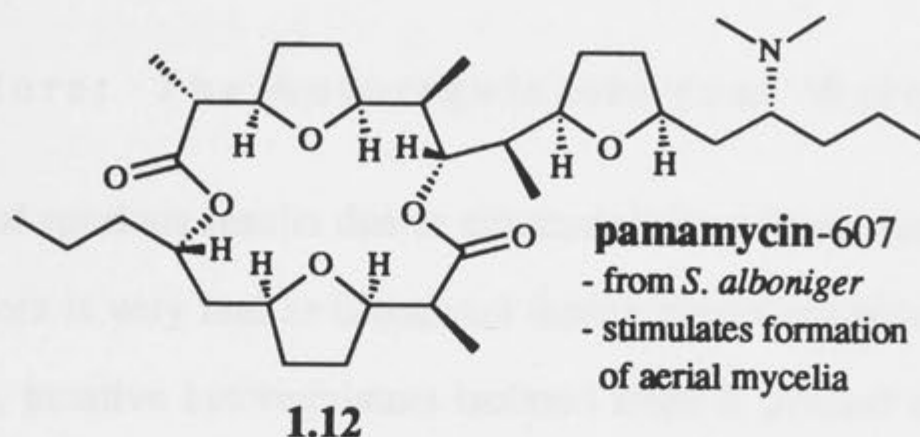
B-factor, 3'-(1-butylphosphoryl)adenosine (**1.11**), was isolated and identified in 1984 as the active factor present in yeast extracts which enabled them to restore rifamycin B production to a non-producing mutant strain of *Nocardia* sp.<sup>38</sup> Isolated or synthetic B-factor (**1.11**) was active in rifamycin B induction at a concentration of 2 ng/mL and could stimulate the formation of 1500 times its mass of the antibiotic.<sup>38</sup> The classification of this response as 'autoregulatory' was confirmed with the isolation of a substance identical or closely related to B-factor (**1.11**) from cultures of the parental *Nocardia* sp.<sup>39</sup>



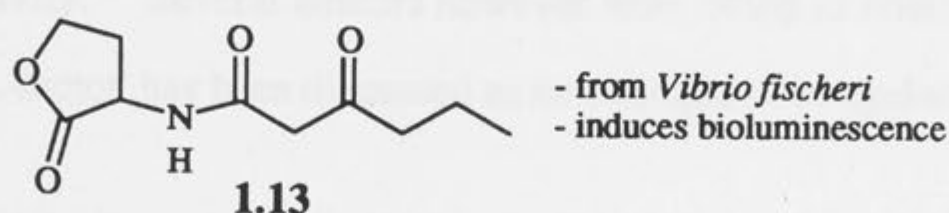
**B-factor**  
- from *Nocardia* sp.  
- induces rifamycin B  
biosynthesis

**1.11**

## 1.4.2 Pamamycin



Studies into aerial mycelia formation by *S. alboniger* revealed the presence of a complex system of specific autoregulatory compounds.<sup>40</sup> Culture extracts contained two unidentified factors which inhibited aerial mycelial growth in addition to a potent aerial mycelia stimulating substance, named 'pamamycin'. Pamamycin was also found to possess antifungal and antibacterial activity. Marumo's group showed that pamamycin was a mixture of more than 8 homologous compounds containing a 16-membered macrodiolide ring, with molecular weights ranging from 593 to 691.<sup>41</sup> The structure of one homologue, pamamycin-607 (**1.12**) (MW 607), was elucidated<sup>41</sup> and further work established the absolute stereochemistry, as depicted above.<sup>42</sup>

1.4.3 An Autoregulator of Bioluminescence in *Vibrio fischeri*

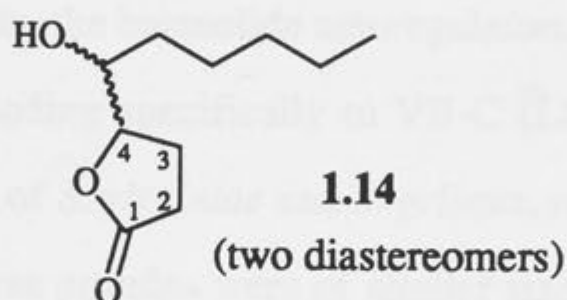
Another interesting example of autoregulation (though not in an actinomycete) is the induction of the enzymes required for bioluminescence (luciferase) in the marine bacterium *Vibrio fischeri* by the homoserine lactone derivative **1.13**.<sup>43</sup> Luciferase is only produced when the concentration of the autoregulator **1.13** in the culture medium reaches a critical level. The autoinducer is species specific and its activity is sensitive to structural alterations.<sup>44</sup> It was thus not surprising that A-factor was found to be



completely inactive as an inducer in this organism despite some structural similarities between the two autoregulators.<sup>44</sup>

#### 1.4.4 L-factors: The Autoregulators that Weren't

The risk of spurious results due to contamination of test samples by extremely active bioregulators is very real as illustrated during the investigation of the so-called 'L-factors' (**1.14**), putative autoregulators isolated from *S. griseus* cultures by Gräfe's research group.<sup>45</sup>



Despite their considerable structural difference from A-factor (**1.1**), the purified metabolites apparently displayed low but significant A-factor-like autoregulatory activity towards leukaemomycin production in mutant indicator strains of *S. griseus* (ca 5% of A-factor's activity).<sup>45</sup> Although Gräfe and coworkers "unambiguously excluded" the occurrence of traces of A-factor (**1.1**) by "careful GC analysis", they subsequently had to concede that the observed biological activity was in fact due to contamination by A-factor, when synthetic samples of the lactones **1.14** failed to show any inducing activity.<sup>46</sup> Several authors however have failed to note the retraction and as late as 1988 'L-factor' has been discussed as an example of a regulatory metabolite.<sup>39</sup>

## 1.5 MECHANISM OF ACTION OF THE BUTANOLIDE AUTOREGULATORS

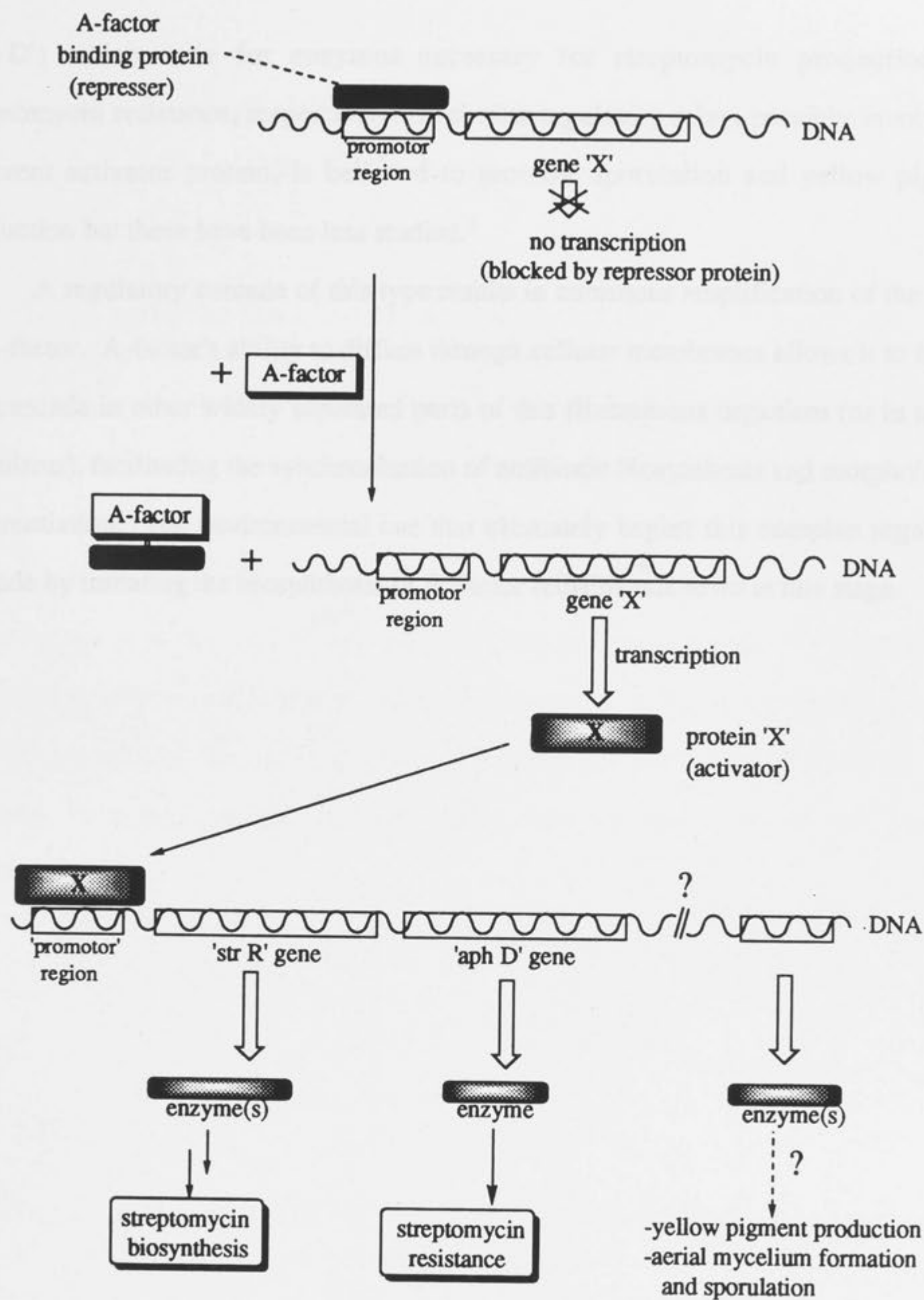
Early reviews of the biological activity of A-factor<sup>5,6</sup> included speculation as to its mode of action but the lack of mechanistic evidence precluded the development of a coherent model to explain both the extreme potency and the diversity of its actions. However, recent investigations have contributed greatly to the understanding of the regulatory cascade initiated by the butanolide autoregulators.

In 1989, proteins binding specifically to VB-C (1.8)<sup>47,48</sup> and A-factor (1.1)<sup>49</sup> were isolated from cultures of *S. virginiae* and *S. griseus*, respectively, with the use of <sup>3</sup>H-labelled substrates. These proteins were of similar size (MW *ca* 30,000) and both are present at levels of about 30-40 molecules per genome.

The supposition that the butanolide autoregulators expressed their regulatory activities through binding with these proteins is supported by studies of an anomalous mutant strain of *S. griseus* which does not require A-factor for streptomycin production or sporulation. Miyake and coworkers found that this mutant strain (*S. griseus* 2247) has a defect in its A-factor-binding protein<sup>50</sup> leading to the conclusion that: "*the binding protein in the wild-type strain acts as a repressor-type regulator for streptomycin production and sporulation and that the repression is released by binding with A-factor*".<sup>50</sup> In mutant strains lacking an intact repressor protein, A-factor can no longer regulate the timing of antibiotic production and sporulation, and consequently these processes occur at a much earlier stage of growth than in the wild-type strain.<sup>50</sup>

Figure 1.1 depicts a model proposed by Horinouchi and Beppu for the regulatory cascade induced by A-factor in *S. griseus* based on extensive genetic analyses.<sup>3</sup>





**Figure 1.1 Model Proposed by Horinouchi and Beppu for the Regulatory Cascade Initiated by A-Factor**

The A-factor-binding protein inhibits the expression of a putative gene 'X', probably by attachment to its promoter region until it is removed by binding with A-factor (1.1) (Fig 1.1). Subsequent transcription of this gene is believed to produce an activating protein (X) that promotes the transcription of further genes ('str R' and

'aph D') which code for enzymes necessary for streptomycin production and streptomycin resistance, respectively. A similar regulatory relay, possibly involving a different activator protein, is believed to promote sporulation and yellow pigment production but these have been less studied.<sup>3</sup>

A regulatory cascade of this type results in enormous amplification of the effect of A-factor. A-factor's ability to diffuse through cellular membranes allows it to initiate this cascade in other widely separated parts of this filamentous organism (or in nearby organisms), facilitating the synchronisation of antibiotic biosynthesis and morphological differentiation. The environmental cue that ultimately begins this complex regulatory cascade by initiating the biosynthesis of A-factor remains unknown at this stage.

The natural A-factor (1.1)<sup>23</sup> and two of its biologically active forms<sup>24,25</sup> following the following scheme in the present microbial biosynthesis. All of the systems, however, follow essentially the same strategy involving the synthesis of a derivative of 3-hydroxy-2-methylbutyrate (1.17a-c) which was then cyclized to the 2-position (Scheme 1.1).



Scheme 1.1 Derivatives of 3-Hydroxy-2-methylbutyrate Utilized in Literature Synthesis of A-factor

Shibata et al.

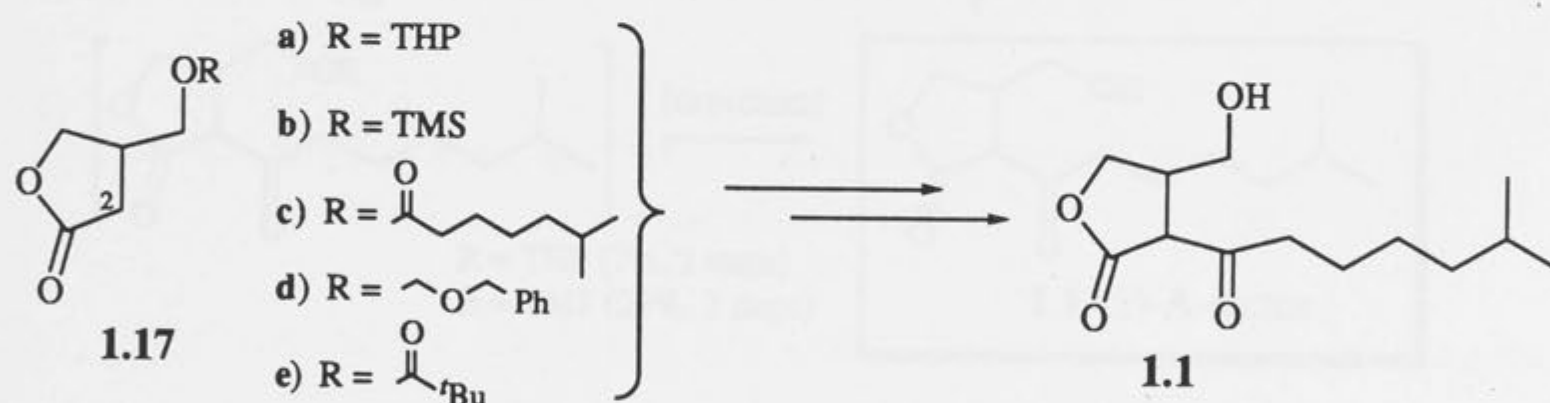
In the first synthesis of natural A-factor (1.1), Shibata and co-workers investigated three of other derivatives (1.17a, 1.17b and 1.17c) as intermediates.<sup>24</sup> They were prepared in four steps from 3-oxo-2-methylbutyrate (1.18) (overall yields 34%, 35% and 41% respectively) which was itself derived from diethyl malonate (1.19) in an 80% overall yield (Scheme 1.2).



## 1.6 SYNTHESSES OF THE BUTANOLIDE AUTOREGULATORS

### 1.6.1 Synthetic Routes to A-factor (1.1)

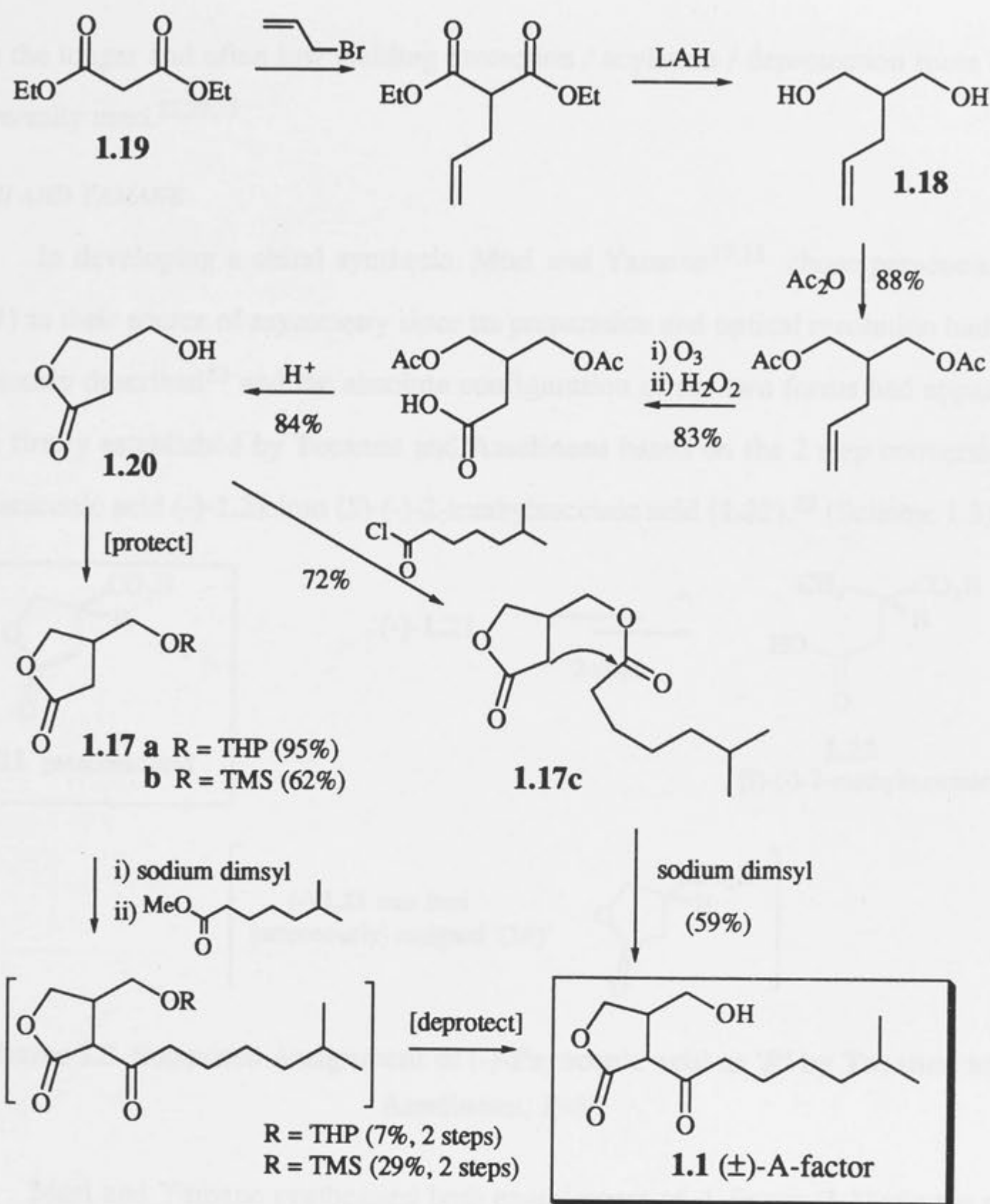
Racemic A-factor (**1.1**) was first synthesised by Khokhlov's research group in 1977<sup>51</sup> to confirm the structure which they had proposed for the autoregulator, based on physico-chemical studies.<sup>9</sup> In 1982, Mori and Yamane reported the synthesis of both optically active forms.<sup>18</sup> Recent years have seen reports of three further syntheses, one for racemic A-factor (**1.1**)<sup>29</sup> and two of optically active forms,<sup>20,52</sup> reflecting the continuing interest in this potent microbial hormone. All of the syntheses, however, follow essentially the same strategy involving the synthesis of a derivative of 3-hydroxymethylbutanolide (**1.17a-e**) which was then acylated in the 2-position (Scheme 1.1).



**Scheme 1.1** Derivatives of 3-Hydroxymethylbutanolide Utilised in Literature Syntheses of A-factor

*KHOKHLOV et al.*

In the first synthesis of racemic A-factor (**1.1**), Khokhlov and coworkers investigated three of these derivatives (**1.17a**, **1.17b** and **1.17c**) as intermediates.<sup>51</sup> They were prepared in four steps from 2-allyl-1,3-propanediol (**1.18**) (overall yields 58%, 38% and 44% respectively) which was itself derived from diethyl malonate (**1.19**) in an undisclosed yield (Scheme 1.2).



**Scheme 1.2** Synthesis of (±)-A-factor by Khokhlov and coworkers

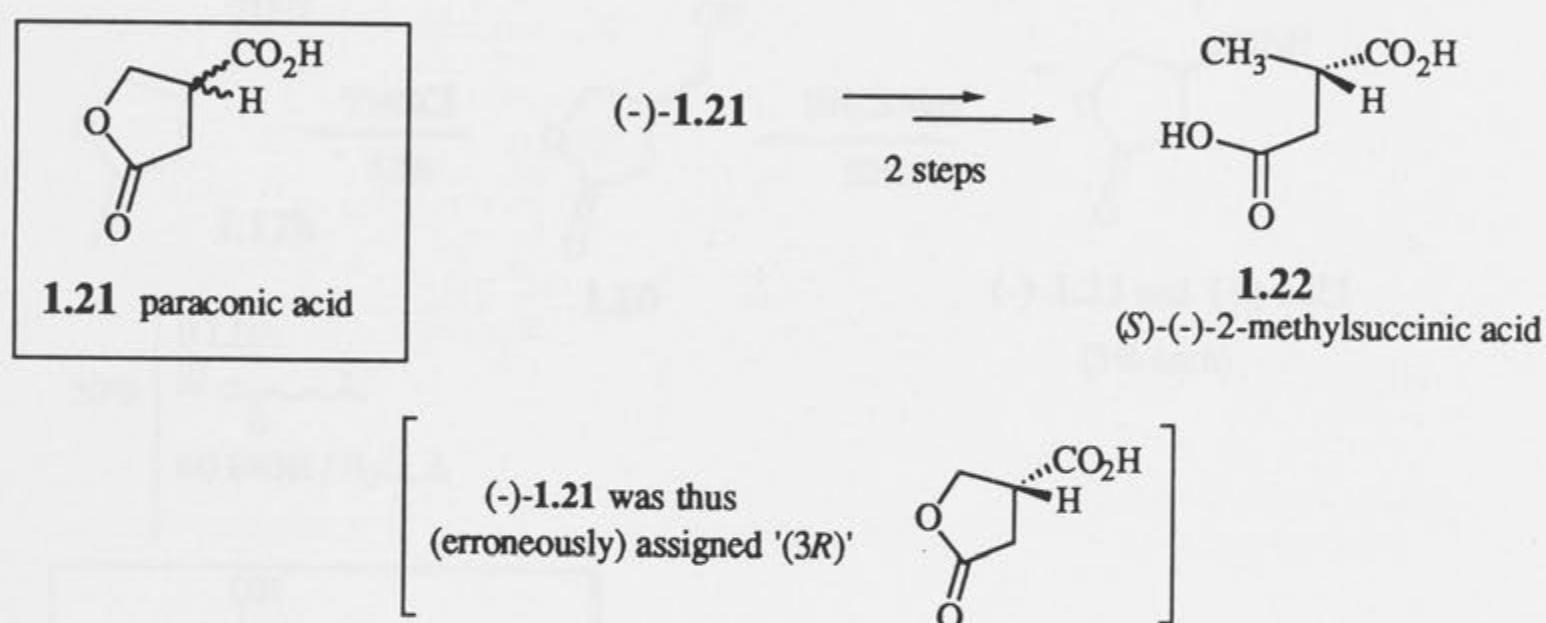
Acylation of the THP- or TMS-protected butyrolactones **1.17a** or **1.17b** (sodium dimsyl / DMSO; methyl 6-methylheptanoate) followed by deprotection gave racemic A-factor (**1.1**) in only low yields (7% and 29% respectively). The yield was improved to 59% with the use of the 6-methylheptanoate ester **1.17c** since the required acylation could be achieved *via* a facile intramolecular O-C acyl transfer and hence no deprotection step was required. Unaccountably, this useful improvement has not been employed in subsequently reported syntheses of racemic A-factor and its analogues,



with the longer and often low yielding protection / acylation / deprotection route being universally used.<sup>25,29,35</sup>

#### MORI AND YAMANE

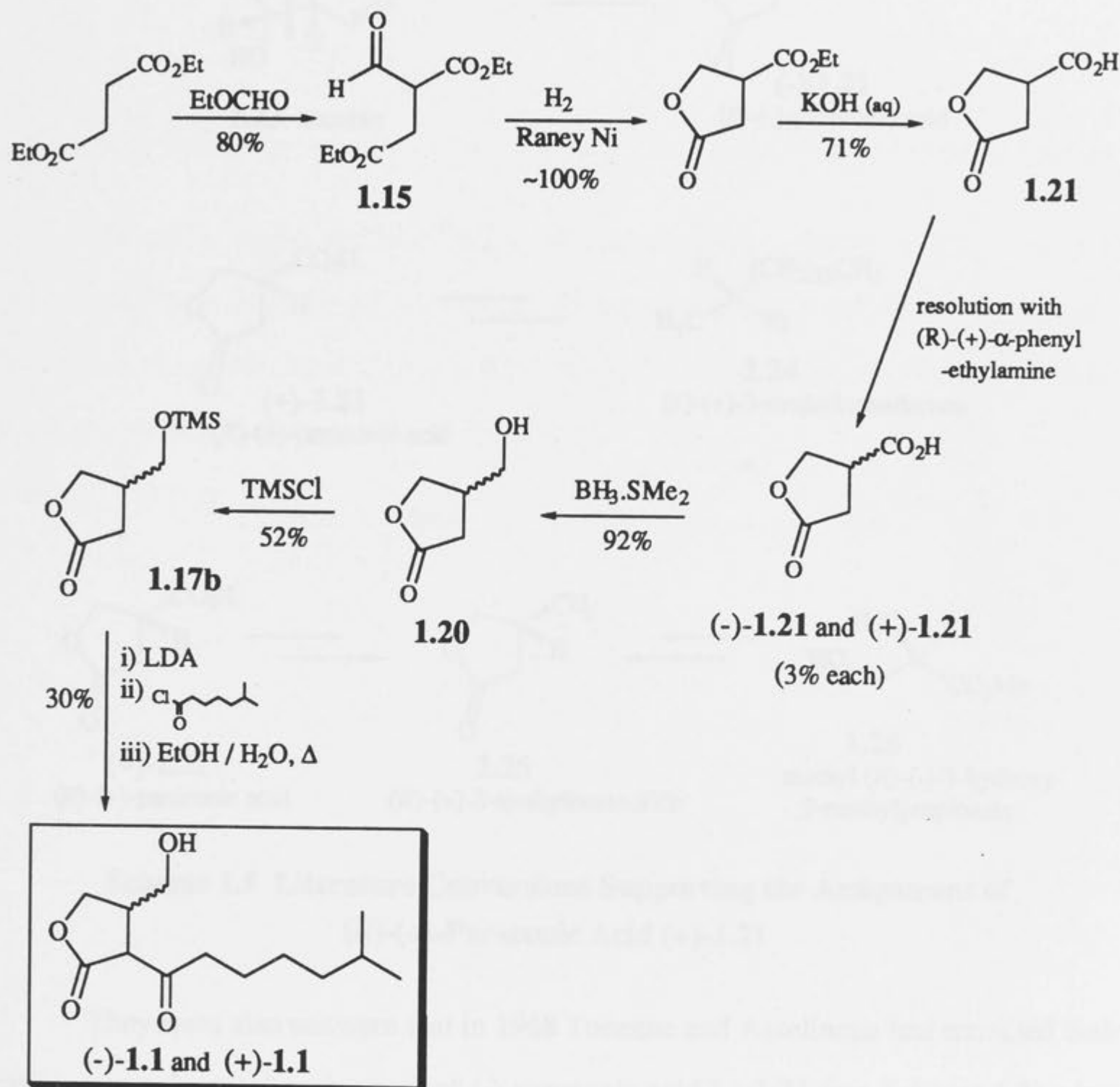
In developing a chiral synthesis, Mori and Yamane<sup>17,18</sup> chose paraconic acid (**1.21**) as their source of asymmetry since its preparation and optical resolution had been previously described<sup>53</sup> and the absolute configuration of the two forms had apparently been firmly established by Tocanne and Asselineau based on the 2 step conversion of (-)-paraconic acid (-)-**1.21** into (*S*)-(-)-2-methylsuccinic acid (**1.22**),<sup>53</sup> (Scheme 1.3).



**Scheme 1.3** Supposed Assignment of (-)-Paraconic acid as '*R*' by Tocanne and Asselineau, 1965

Mori and Yamane synthesised both enantiomers of A-factor (**1.1**) *via* the route outlined in Scheme 1.4, in which the optically pure paraconic acid enantiomers (-)-**1.21** and (+)-**1.21**, obtained by a laborious and inefficient resolution with (*R*)-(+)- $\alpha$ -phenylethylamine, were separately reduced to 3-hydroxymethylbutanolide (**1.20**) and then TMS-protected to form the intermediate **1.17b** previously used by Khokhlov's group. The overall yield of the final acylation and deprotection steps (30%) represented no significant improvement over the Russian synthesis, despite the use of LDA, low temperatures (-78°C, 1 h) and the more active acid chloride as the acylating agent. Although the overall yield of optically pure products (-)-**1.1** and (+)-**1.1** from diethylsuccinate was minuscule (*ca* 0.2%), the synthesis served to demonstrate that

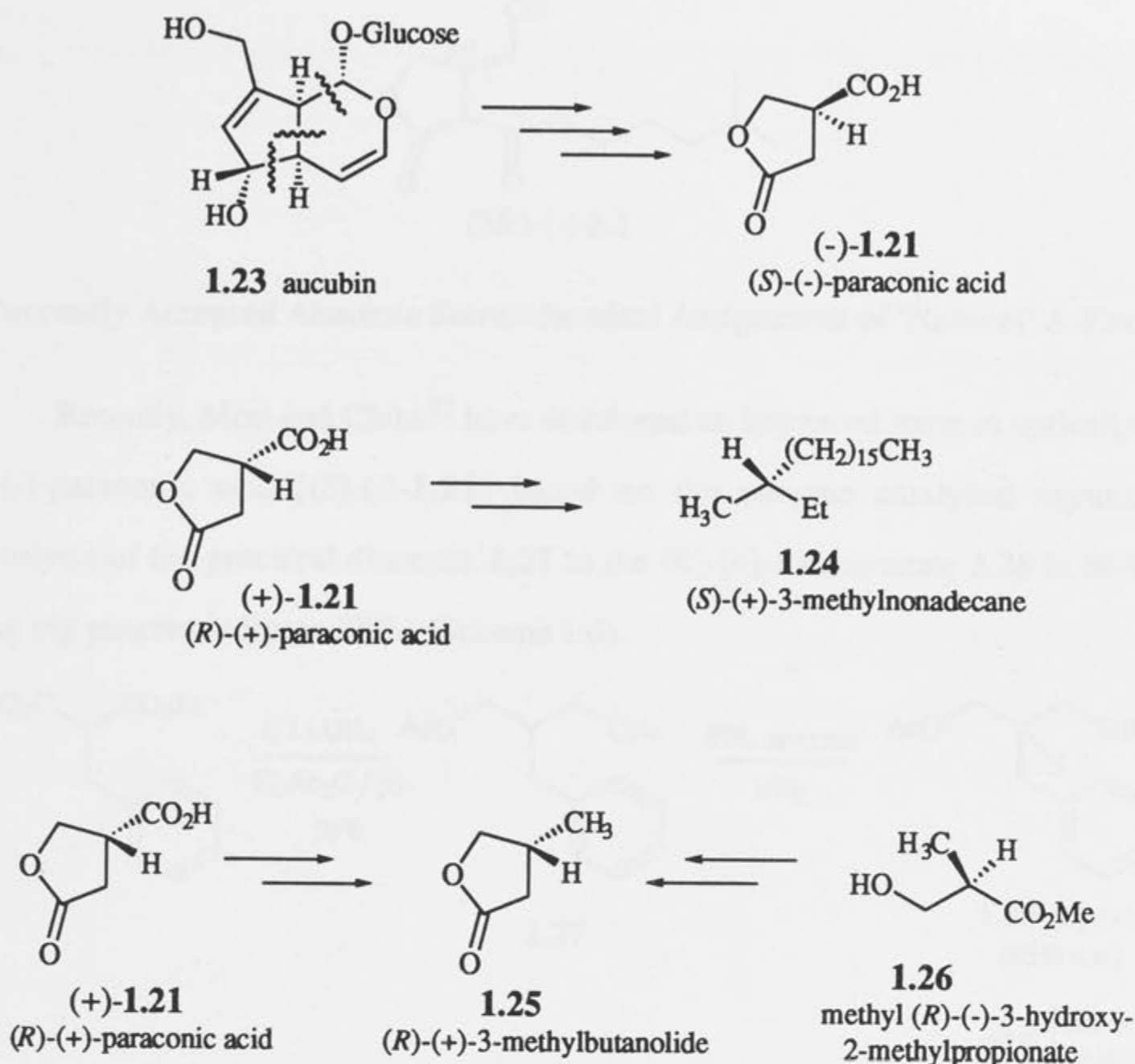
(-)-A-factor (-)-**1.1**, corresponding to the natural metabolite, could be prepared from (-)-paraconic acid (-)-**1.21**.



**Scheme 1.4** Synthesis of Optically Active Forms of A-factor by Mori and Chiba

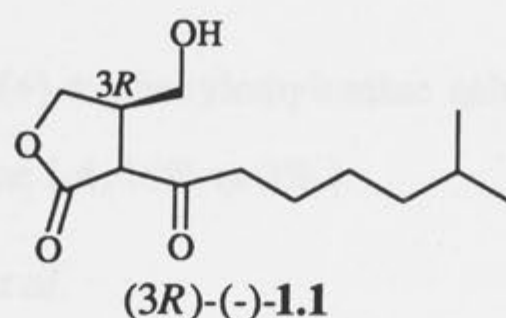
Relying on the purported assignment of (-)-paraconic acid (-)-**1.21** as 'R'<sup>53</sup> (see Scheme 1.3), Mori assigned '3S' stereochemistry to the natural autoregulator (-)-**1.1**.<sup>17,18</sup> Unfortunately, Mori and Yamane were unaware at that time of other reputable reports supporting the opposite stereochemical assignment (S) for (-)-paraconic acid (-)-**1.21** based on its formation from aucubin (**1.23**),<sup>54,55</sup> as shown in Scheme 1.5.





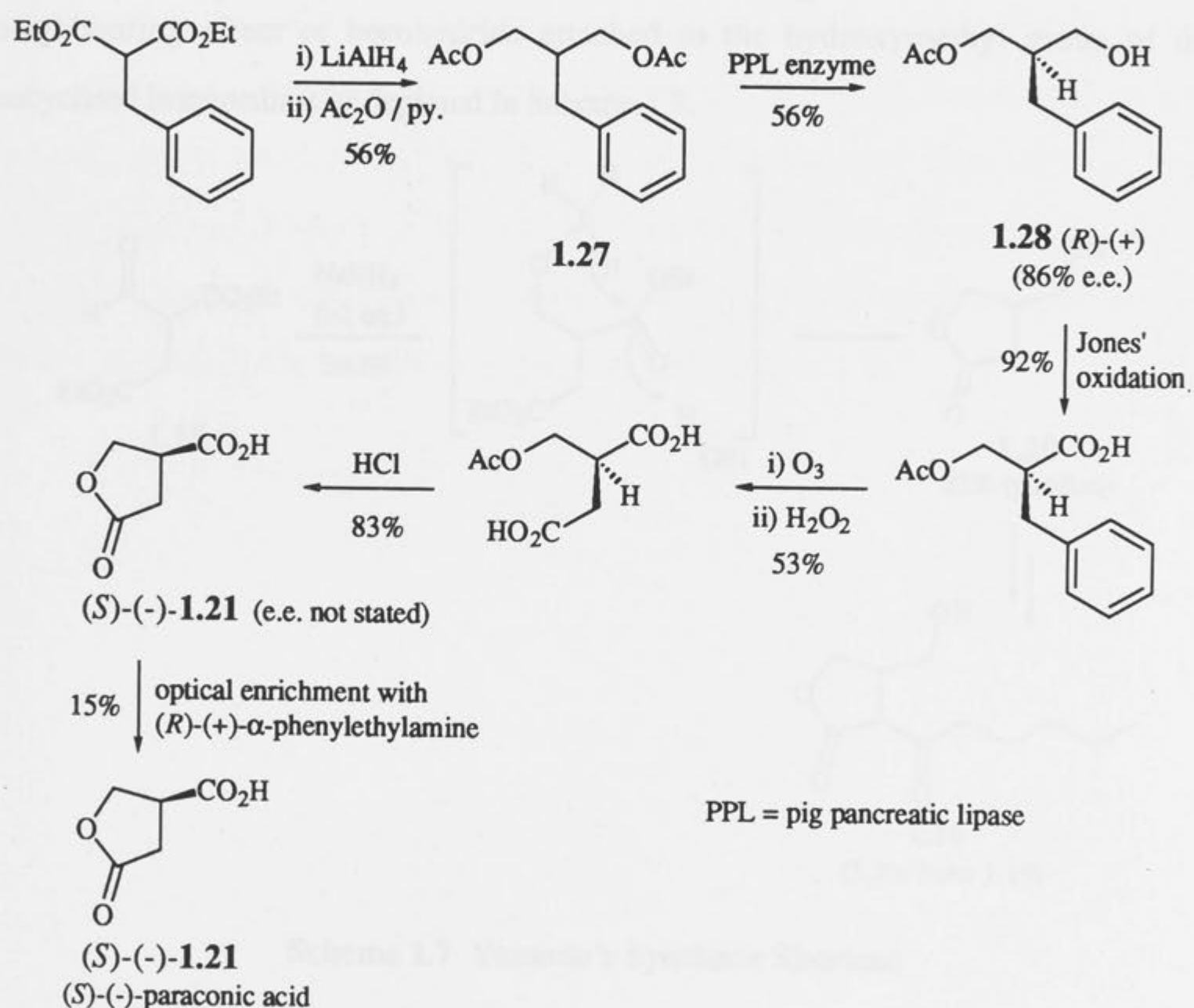
**Scheme 1.5 Literature Conversions Supporting the Assignment of (*R*)-(+)-Paraconic Acid (+)-1.21**

They were also unaware that in 1968 Tocanne and Asselineau had retracted their earlier stereochemical assignment of (-)-paraconic acid (-)-1.21 (see Scheme 1.3) when they found that the (+)-enantiomer (+)-1.21 could be converted in nine steps to (*S*)-(+)-3-methylnonadecane 1.24, supporting its reassignment as *R* (Scheme 1.5).<sup>56</sup> In 1983, Mori revised his assignment of the absolute configuration of 'natural' (-)-A-factor to 3*R* (depicted below) when he too confirmed that the original stereochemical assignment of the optically active paraconic acid enantiomer (-)-1.21 by Tocanne and Asselineau<sup>53</sup> (see Scheme 1.3) was in error.<sup>19</sup> He showed that (+)-paraconic acid (+)-1.21 could be converted in 3 steps to (*R*)-(+)-3-methylbutanolide (1.25) whose stereochemical assignment was in turn verified by its synthesis in 5 steps from enzymatically derived methyl (*R*)-(-)-3-hydroxy-2-methylpropionate (1.26) (Scheme 1.5).



### Currently Accepted Absolute Stereochemical Assignment of 'Natural' A-Factor

Recently, Mori and Chiba<sup>57</sup> have developed an improved route to optically pure (S)-(-)-paraconic acid [(S)-(-)-1.21] based on the enzyme catalysed asymmetric hydrolysis of the prochiral diacetate 1.27 to the (R)-(+)-monoacetate 1.28 in 86% e.e. using pig pancreatic lipase (PPL) (Scheme 1.6).



**Scheme 1.6 Preparation of (S)-(-)-Paraconic acid (S)-(-)-1.21 by Mori and Chiba Utilising Enzymatic Hydrolysis**

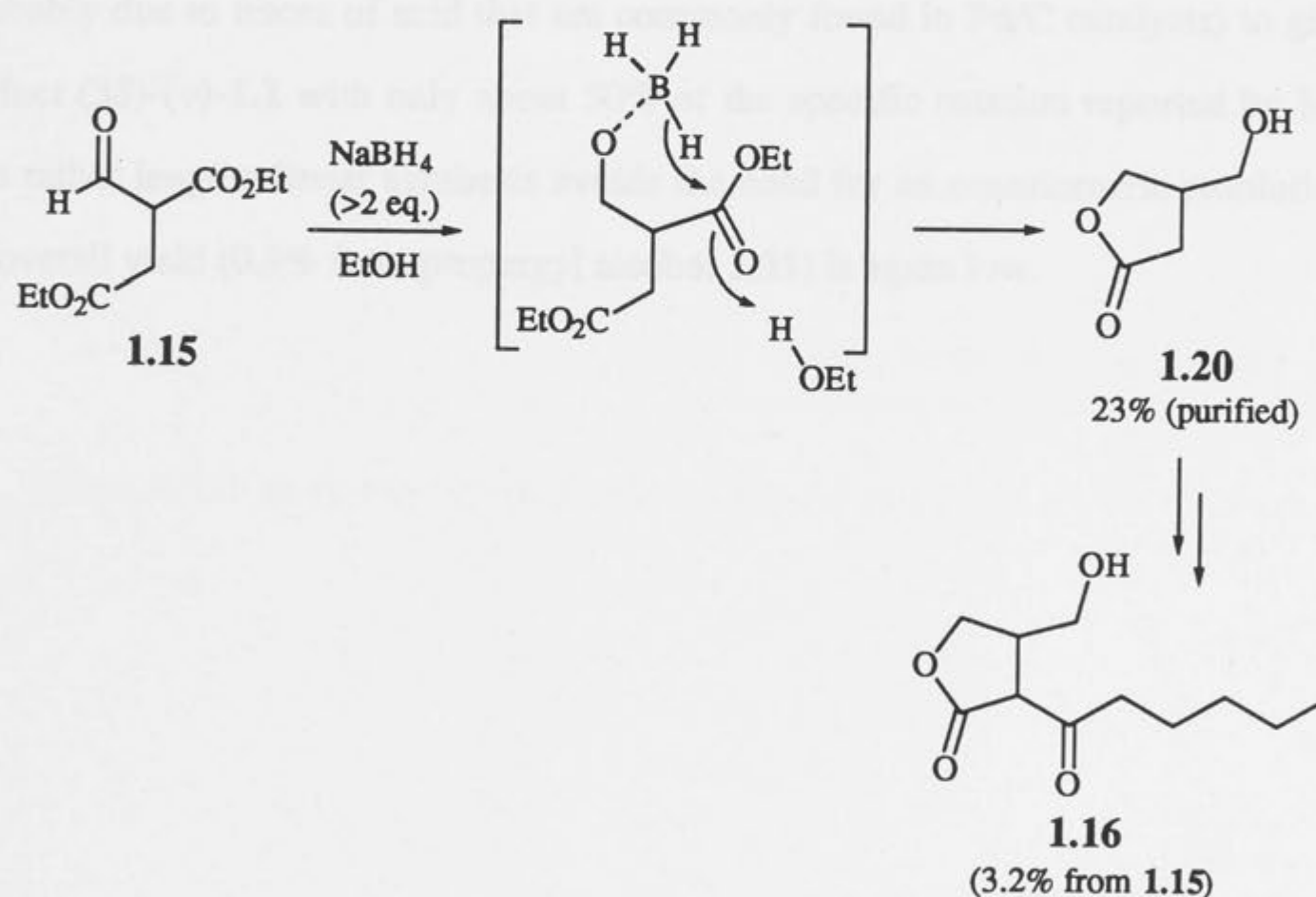
Conversion to paraconic acid 1.21 via the method outlined in Scheme 1.6 gives a product enriched (e.e. not stated) in the (S)-(-)-enantiomer (S)-(-)-1.21, which can be



further enriched as the (*R*)-(+)- $\alpha$ -phenylethylamine salt. This is more efficient than Mori's previous route (Scheme 1.4, 15% vs 3% ).

*MODIFICATION OF YAMADA et al.*

In 1987 Yamada's group utilised a modification of Mori's original route (see Scheme 1.4) for the synthesis of racemic VB-C (**1.8**).<sup>25</sup> Since resolution was not required they were able to considerably shorten the synthesis by the direct one-step reduction and cyclisation of diethylformylsuccinate (**1.15**) to 3-hydroxymethylbutanolide (**1.20**) using sodium borohydride in ethanol (Scheme 1.7). This unusual reduction of an ester group by borohydride was attributed to the neighbouring effect of borohydride attached to the hydroxymethyl group of the uncyclised intermediate as depicted in Scheme 1.7.



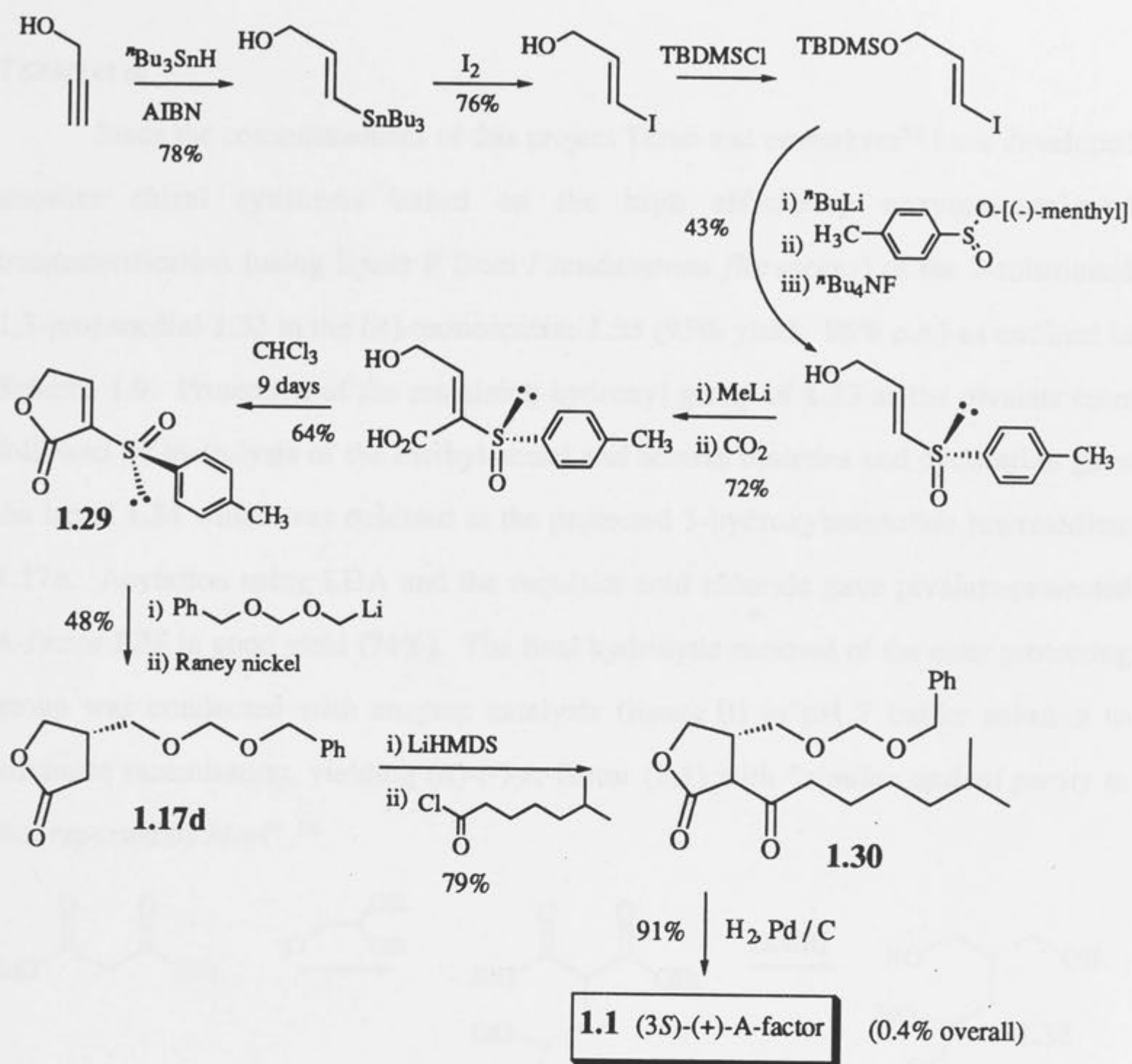
**Scheme 1.7 Yamada's Synthetic Shortcut**

Although this method shortened the route to racemic butanolides, the yield of the purified lactone intermediate **1.20** was quite modest (23%) and thus the overall yield of butanolide autoregulators and analogues was still low (*e.g.* **1.16**, 3.2% from diethylformylsuccinate, **1.15**).

*POSNER et al.*

Posner and coworkers took a different approach to Mori in their frequently overlooked<sup>29,52</sup> asymmetric synthesis of the 'unnatural' 3*S* enantiomer of A-factor (3*S*)-(+)-**1.1** (Scheme 1.8).<sup>20</sup> Their chiral benzyloxymethyl (BOM) protected 3-hydroxymethylbutanolide intermediate (*S*)-(+)-**1.17d** was prepared in 87% e.e. by conjugate addition of a BOM-protected hydroxymethyl carbanion equivalent (PhCH<sub>2</sub>OCH<sub>2</sub>OCH<sub>2</sub>Li) to the enantiomerically pure sulfinylbutenolide **1.29**, followed by reductive desulfinylation with Raney nickel. Subsequent acylation utilising lithium hexamethyldisilazide (LiHMDS) as base was reported to give an improved yield of the protected A-factor **1.30** compared to previous analogues acylations (see Scheme 1.2 and 1.4). Unfortunately, the final hydrogenolysis step involved partial racemisation (probably due to traces of acid that are commonly found in Pd/C catalysts) to give the product (3*S*)-(+)-**1.1** with only about 50% of the specific rotation reported by Mori.<sup>18</sup> This rather lengthy linear synthesis avoids the need for an enantiomeric resolution but the overall yield (0.4% from propargyl alcohol **1.31**) is again low.

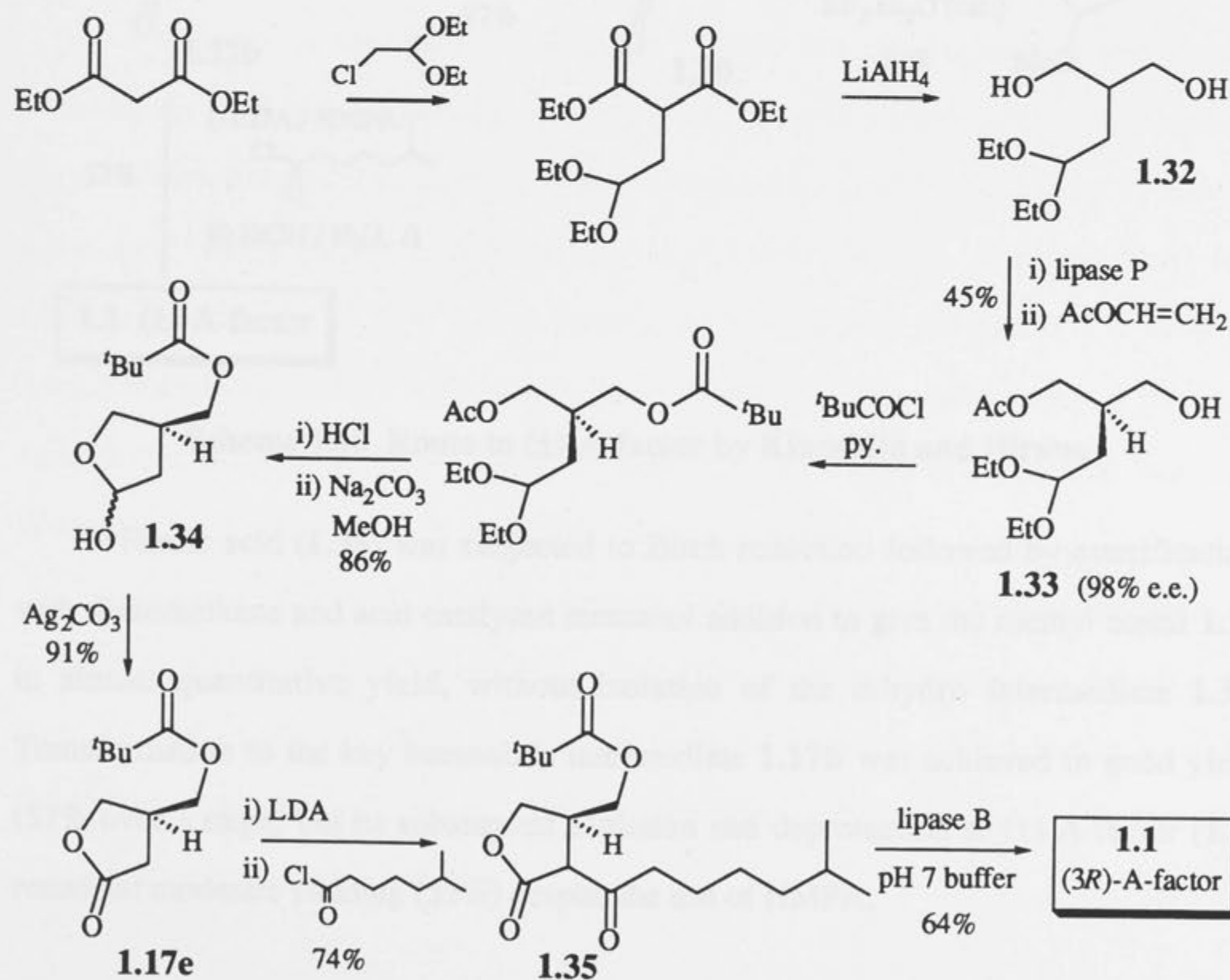




Scheme 1.8 Asymmetric Synthesis of (3S)-(+)-A-Factor by Posner *et al.*

TERAO *et al.*

Since the commencement of this project Terao and coworkers<sup>52</sup> have developed another chiral synthesis based on the high efficiency enzyme-catalysed transesterification (using lipase P from *Pseudomonas fluorescens*) of the 2-substituted 1,3-propanediol **1.32** to the (*R*)-monoacetate **1.33** (95% yield; 98% e.e.) as outlined in Scheme 1.9. Protection of the remaining hydroxyl group of **1.33** as the pivalate ester followed by hydrolysis of the diethyl acetal and acetate moieties and cyclisation gave the lactol **1.34** which was oxidised to the protected 3-hydroxybutanolide intermediate **1.17e**. Acylation using LDA and the requisite acid chloride gave pivalate-protected A-factor **1.35** in good yield (74%). The final hydrolytic removal of the ester protecting group was conducted with enzyme catalysis (lipase B) in pH 7 buffer solution to minimise racemisation, yielding (*R*)-(-)-A-factor (**1.1**) with “similar optical purity to that reported by Mori”.<sup>18</sup>

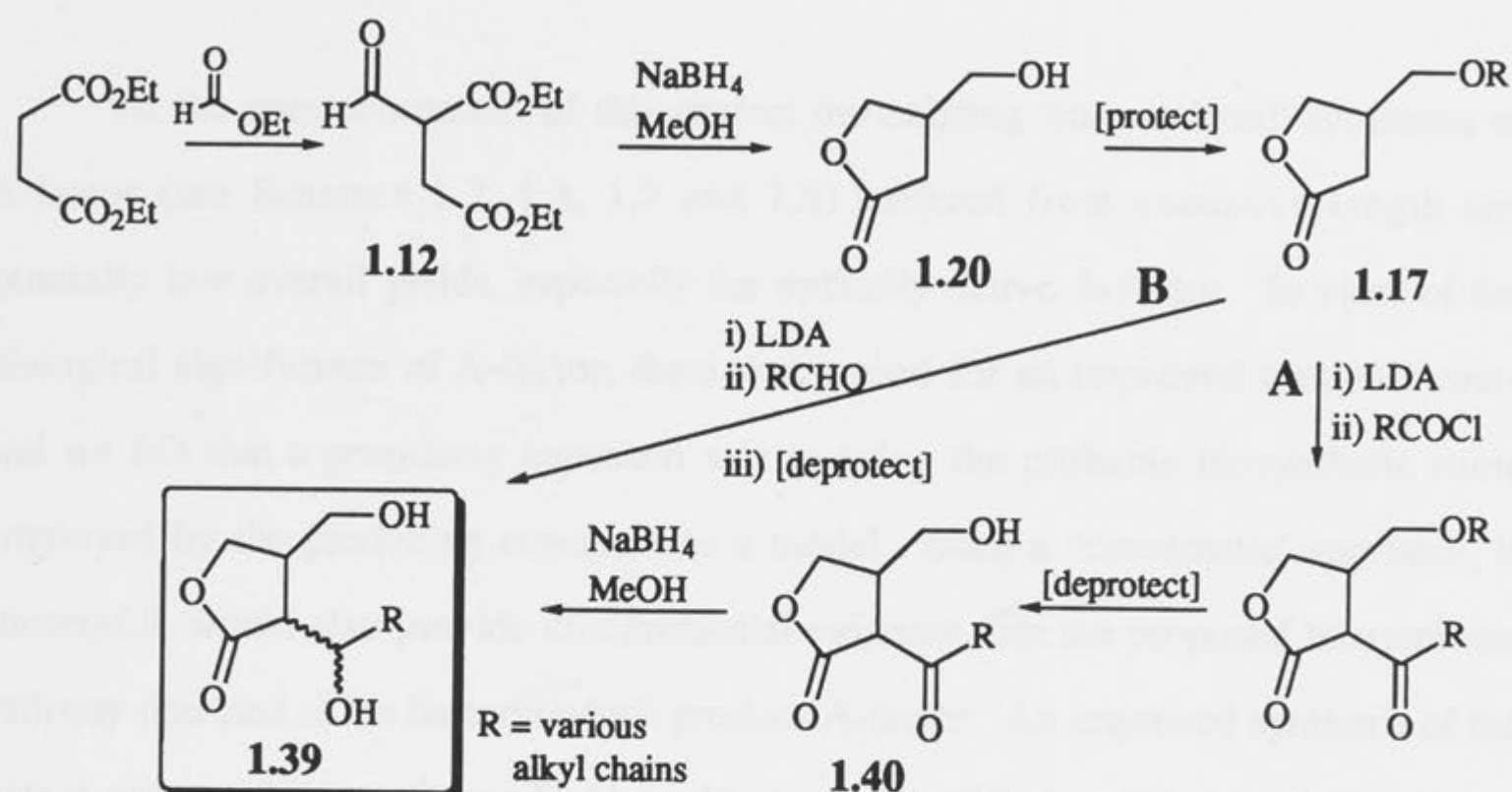


Scheme 1.9 Route to (*3R*)-A-Factor by Terao *et al.* Utilising Enzymatic Hydrolyses





## 1.6.2 Syntheses of the 6-Hydroxybutanolide Autoregulators



Scheme 1.11 General Literature Routes to 6-Hydroxybutanolides

Syntheses of the racemic 6-hydroxybutanolide autoregulators and related homologues **1.39** (R = various alkyl chains) have generally been achieved *via* sodium borohydride reduction of the corresponding 6-keto compounds **1.40** (Scheme 1.11, route A), prepared using Yamada's modified version of Mori's synthesis, discussed above (see Scheme 1.7).<sup>25,30,35</sup> A few examples also exist of direct alkylation of protected 3-hydroxybutanolide **1.17** with aldehydes to form 6-hydroxybutanolides **1.39**, after deprotection (Scheme 1.11, route B).<sup>47,58</sup> The efficiencies and stereochemical outcomes of these methods are discussed in Chapter 6 (Section 6.3).

Recently, Mori and Chiba reported the preparation of 'nature identical' optically active VB-A (**1.6**), VB-B (**1.7**) and VB-C (**1.8**) *via* sodium borohydride reduction of their chiral 6-keto equivalents, themselves prepared in the same manner as optically active A-factor (**1.1**, see Scheme 1.4).<sup>30</sup> This established their absolute configuration at C3 as *R*, in common with A-factor.



## 1.7 PROJECT AIMS

At the commencement of this project the existing 'conventional' syntheses of A-factor (see Schemes 1.2, 1.4, 1.7 and 1.8) suffered from excessive length and generally low overall yields, especially for optically active A-factor. In view of the biological significance of A-factor, there was a need for an improved synthetic route and we felt that a promising approach was to using the probable biosynthetic route employed by the producing organism as a model. Such a 'biomimetic' approach, if successful, would also provide circumstantial evidence that the proposed biosynthetic pathway operated in the bacteria which produce A-factor. An improved synthesis of the potent autoregulator A-factor (**1.1**) could also be modified to improve access to the expanding array of 6-hydroxy autoregulators **1.2-1.10**. In the following chapter our proposal for the biosynthesis of A-factor is outlined and our strategy for the development of a biomimetic synthesis is defined.

## CHAPTER TWO

### BIOMIMETIC STRATEGY

*Lord, I fall upon my knees  
And pray that all my syntheses  
May no longer be inferior  
To those conducted by bacteria.*

D. E. Eveleigh  
Scientific American, 1981, 245, 120



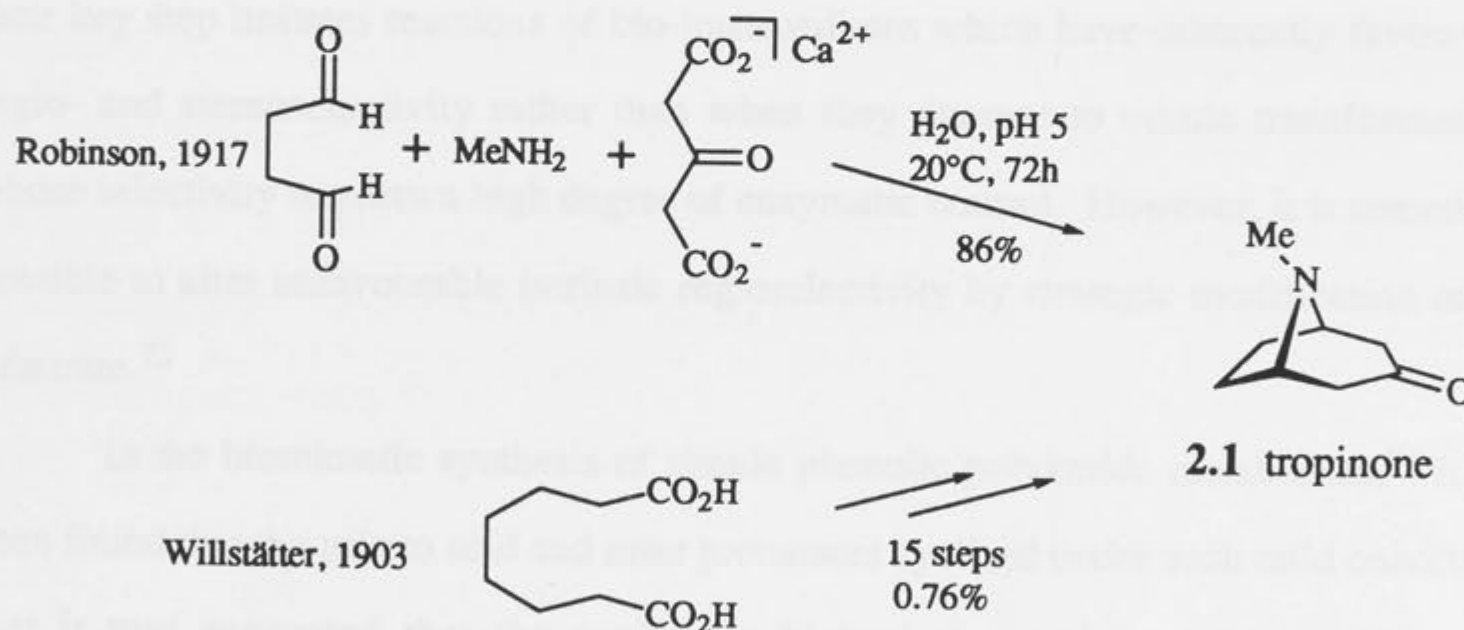
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## 2.1 BIOMIMETIC SYNTHESIS: A PERSPECTIVE

'Biomimetic chemistry' is a term coined by Breslow<sup>59</sup> in 1972 to describe "the branch of organic chemistry which attempts to imitate natural reactions and enzymic processes as a way to improve the power of organic chemistry". 'Biomimetic synthesis', formerly known as 'biogenetic-type' synthesis, has been defined by van Tamelen as the design and execution of laboratory synthesis based (at least in key aspects) upon established or presumed biochemical transformations.<sup>60,61</sup>

The classic synthesis of tropinone (2.1) by Robinson<sup>62,63</sup> (Scheme 2.1) in 1917 is often regarded as the archetypal example of this approach,<sup>64,65</sup> but it is clear that Robinson's biosynthetic proposals,<sup>66</sup> rather than being used in the design of the synthesis, were inspired by the synthetic achievement.



Scheme 2.1

Nevertheless, comparison of this remarkable synthesis with the previous lengthy and laborious conventional route used by Willstätter,<sup>67</sup> provided an early demonstration of the potential of this style of organic synthesis. In recent decades, the application of biomimetic principles to the planning of syntheses has expanded greatly due to both the vast improvement in the understanding of biosynthetic pathways and to the increasing sophistication of organic synthetic methods. Considerable advances have been reported

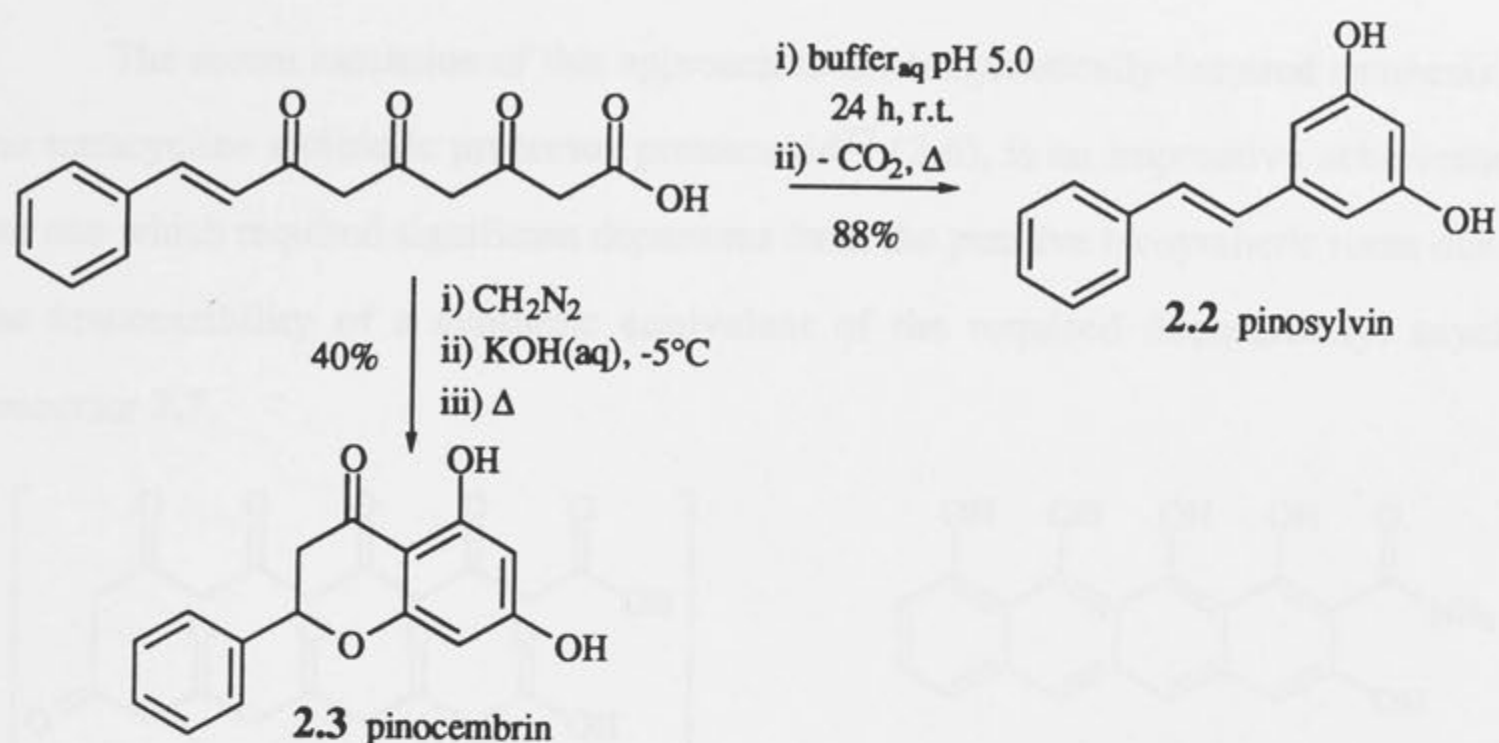


in the development of biomimetic syntheses in the areas of marine natural products,<sup>68</sup> alkaloids,<sup>69,70</sup> polyketide metabolites<sup>71</sup> and triterpenoids.<sup>72</sup>

The transformations known to be utilised by Nature for the biosynthesis of natural products often provide good models on which to base syntheses since their success is precedented, albeit with enzyme assistance. Conversely, in cases where the biosynthesis is less well established, a facile biomimetic synthesis can provide some support for a postulated biosynthetic route and guide further biosynthetic study.

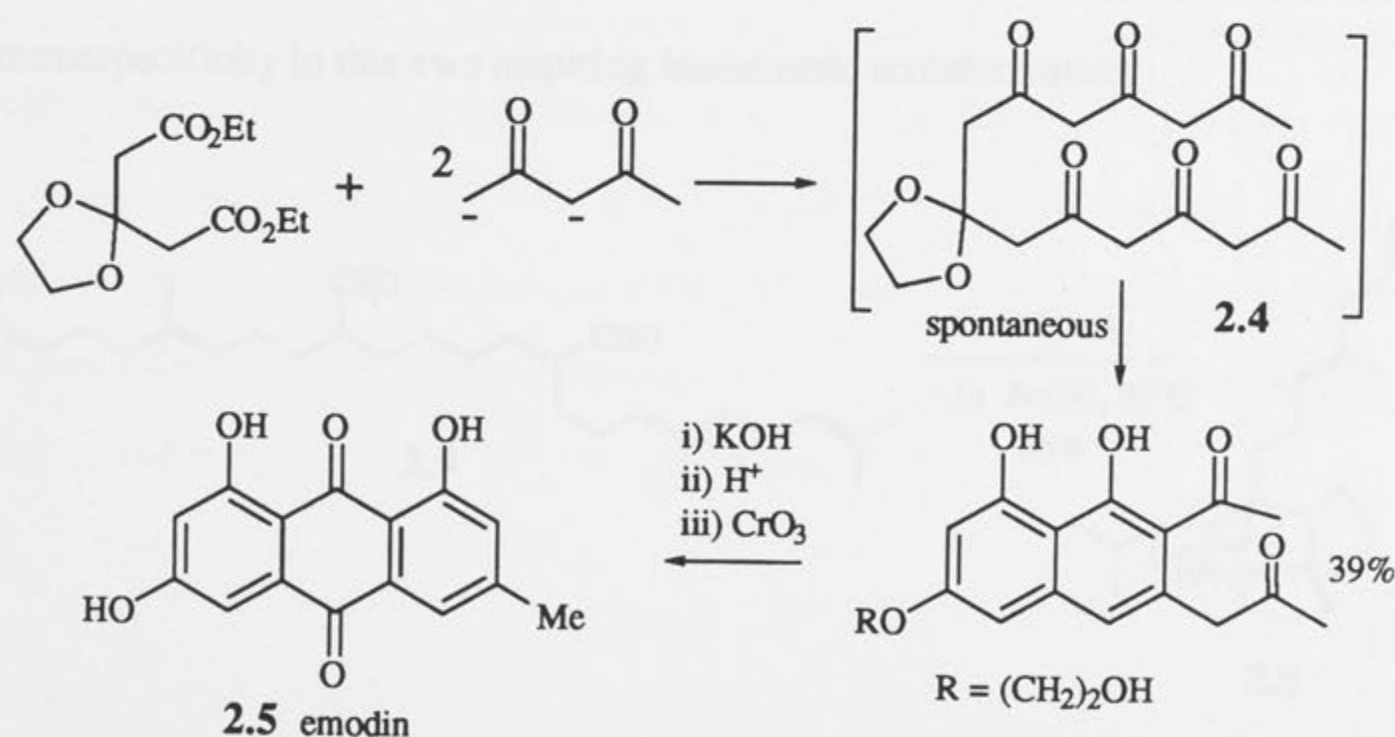
Biomimetic syntheses are generally characterised by a small number of steps and a high total yield.<sup>65</sup> They typically utilise a key, biologically inspired transformation (*e.g.* a key bond formation or cyclisation) which constitutes the focal point of the synthetic route. The preparation of the required intermediate for this step and subsequent elaboration to the natural product (if required), is often accomplished by standard synthetic means. Biomimetic syntheses are generally most successful when their key step imitates reactions of bio-intermediates which have inherently favourable regio- and stereoselectivity rather than when they attempt to mimic transformations whose selectivity requires a high degree of enzymatic control. However, it is sometimes possible to alter unfavourable intrinsic regioselectivity by strategic modification of the substrate.<sup>73</sup>

In the biomimetic synthesis of simple phenolic polyketide metabolites,<sup>74</sup> it has been found that the triketo acid and ester precursors cyclised under such mild conditions that it was suggested that the analogous biological reactions may not be under enzymatic control.<sup>75</sup> The selective use of two facile modes of cyclisation achieved the efficient biomimetic syntheses of the plant metabolites pinosylvin (2.2) and pinocembrin (2.3).<sup>74</sup>



Scheme 2.2

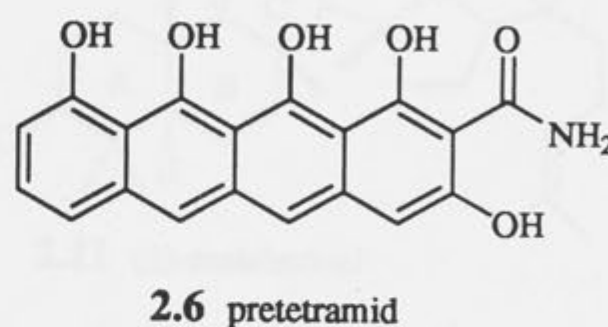
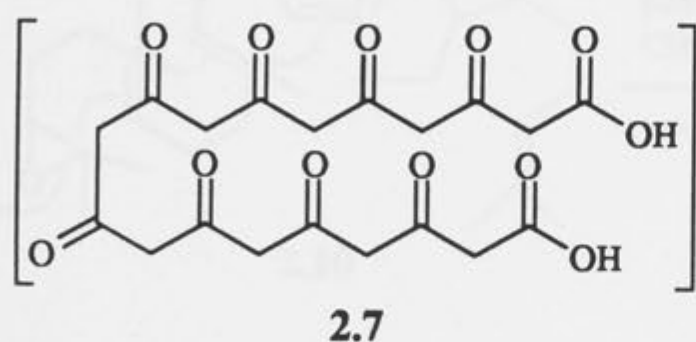
However, for more complex polyketide targets, the preparation of the required lengthy oligocarbonyl precursors and the control of their cyclisation becomes a formidable problem, suggesting that the enzymes involved in this process *in vivo* perform a vital role in the stabilisation and control of the cyclisation regiochemistry of the precursors. For symmetrical heptaketides, Harris and coworkers used strategic protection of one carbonyl group of the heptaketide equivalent 2.4 to achieve spontaneous regiospecific cyclisation, giving exceptionally direct access to emodin (2.5) and related metabolites.<sup>76</sup>



Scheme 2.3

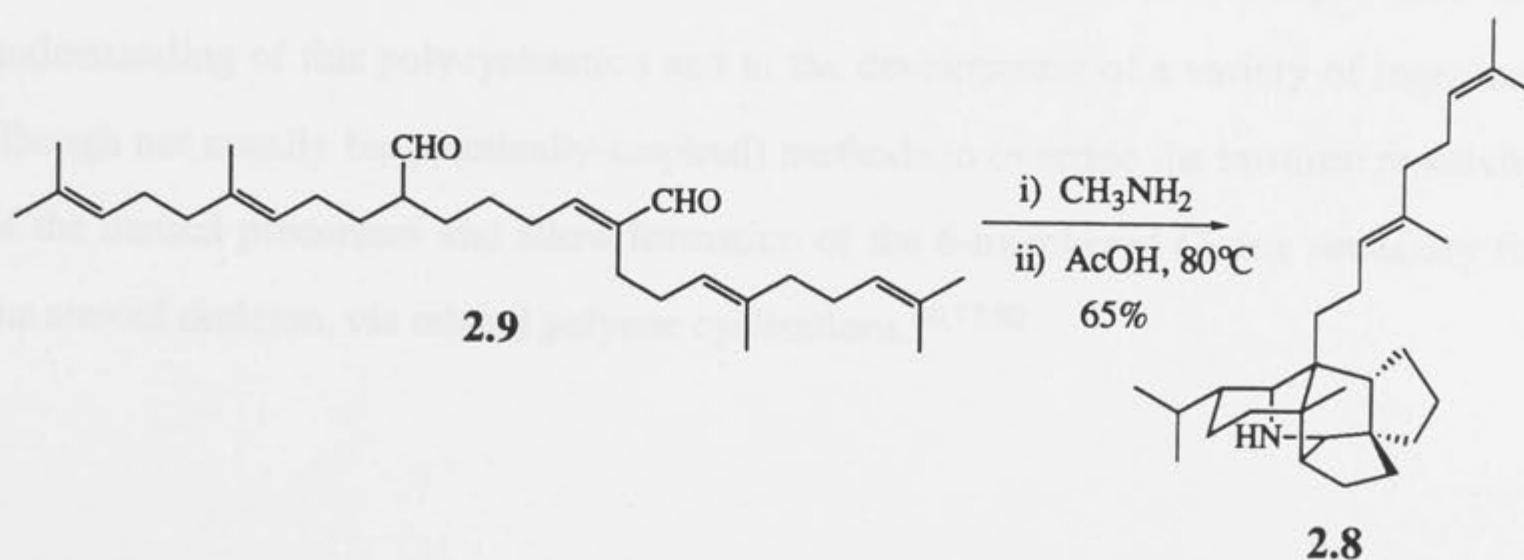


The recent extension of this approach to the biogenetically-inspired synthesis of the tetracycline antibiotic precursor pretetramid<sup>77</sup> (2.6), is an impressive achievement but one which required significant departures from the putative biosynthetic route due to the inaccessibility of a synthetic equivalent of the required decacarbonyl acyclic precursor 2.7.



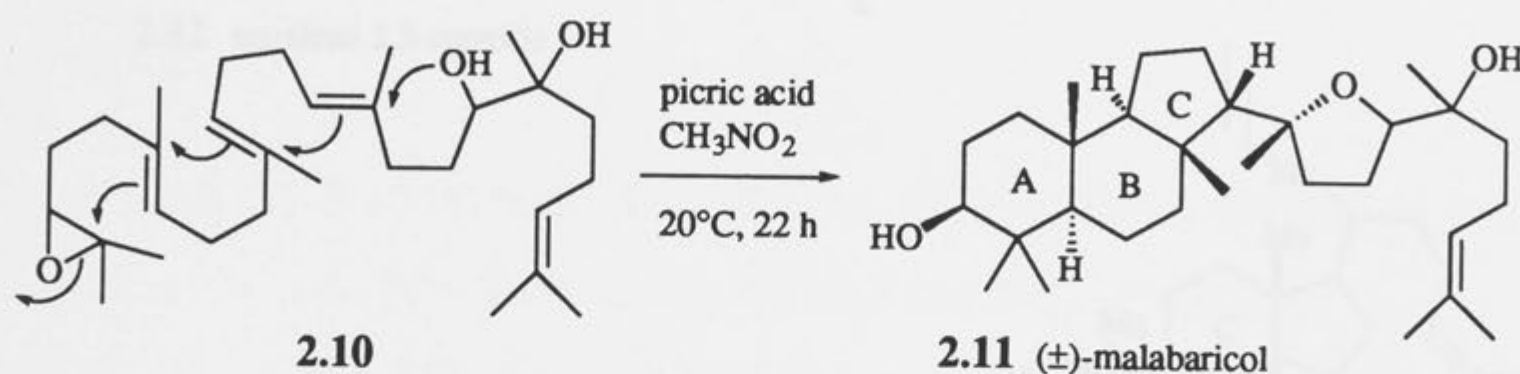
In other biosynthetic systems, key precursors have been found to be stable *in vitro* and display quite amazing inherent regio- and stereospecificity upon cyclisation, despite their considerable size, and have formed the basis of some remarkable biomimetic syntheses.

The recent report of the biomimetic synthesis of the pentacyclic skeleton of the Daphniphyllum alkaloids **2.8** in one step from the acyclic squalene derivative **2.9** provides an extreme example of favourable intrinsic reactivity in a putative biological precursor (Scheme 2.4).<sup>78</sup> Five rings and seven  $\sigma$ -bonds are created with complete diastereospecificity in this awe inspiring biomimetic transformation.



### Scheme 2.4

The diol **2.10** obtained from squalene 2,3-epoxide (**2.12**), cyclises under mildly acidic conditions to give the tetracyclic natural triterpenoid malabaricol (**2.11**) (Scheme 2.5, 7% yield) with concurrent diastereospecific formation of six asymmetric centres.<sup>79</sup>

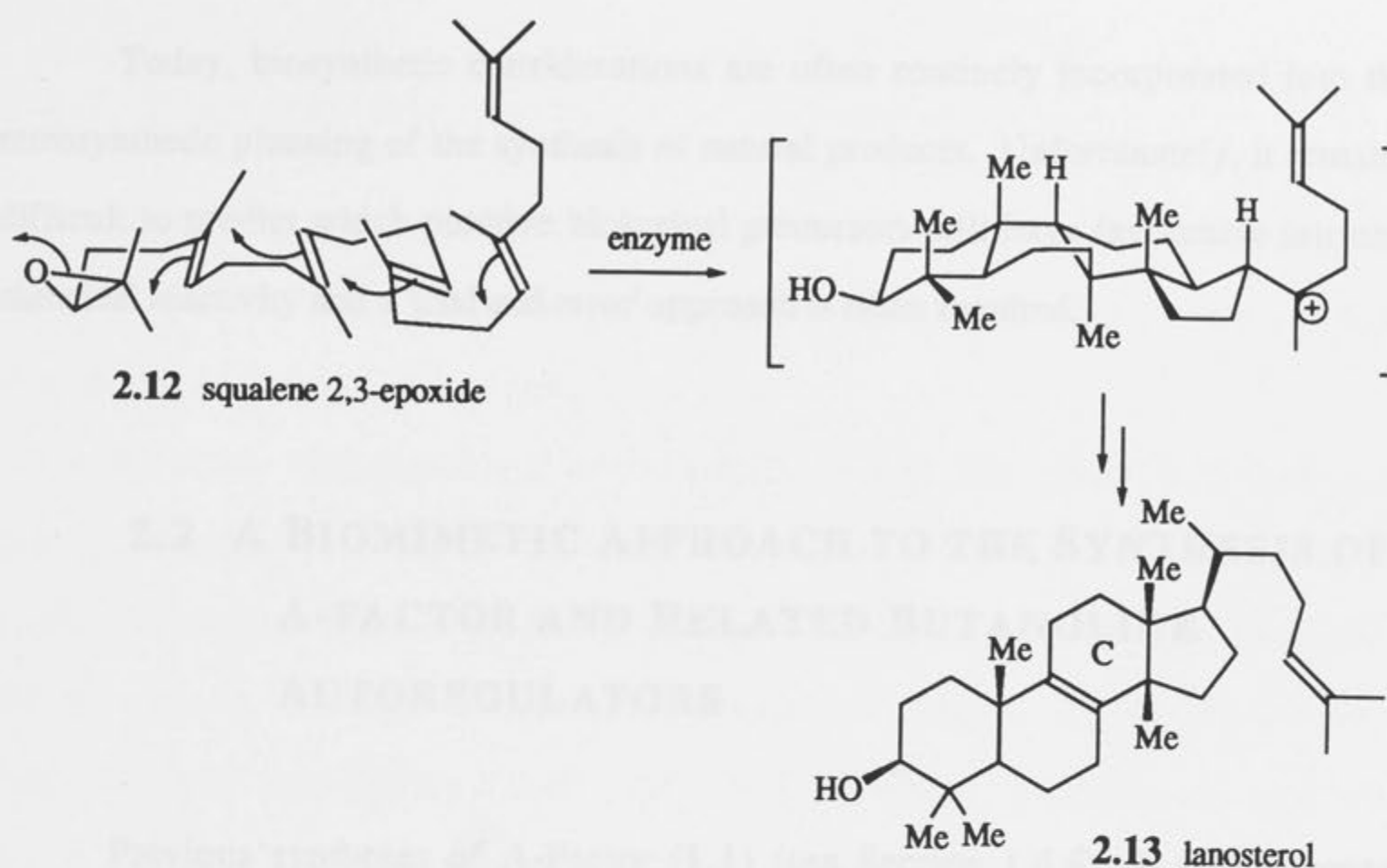


Scheme 2.5

Of course the exceptionally favourable non-enzymatically controlled reactivity demonstrated in the preceding syntheses is unusual and it is sometimes found that key biosynthetic steps are not readily amenable to chemical analogy.

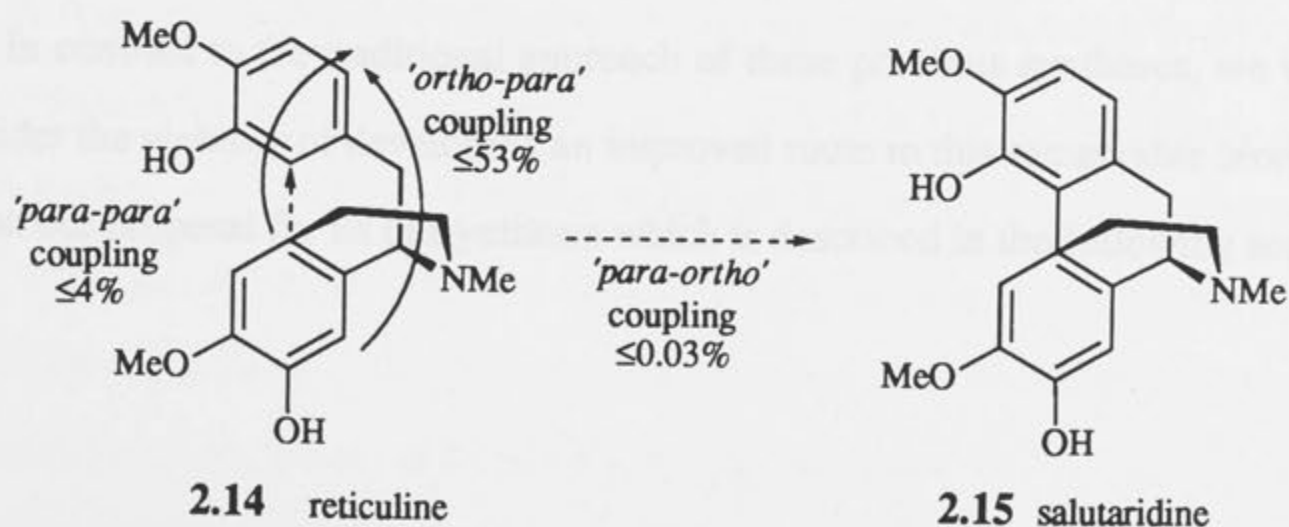
For example, it is ironic that the inherent chemical tendency of squalene 2,3-epoxide derivatives to cyclise to triterpenoids with a 5-membered C-ring, as demonstrated in the remarkable biomimetic formation of malabaricol (**2.11**) (see Scheme 2.5), represents one of the major obstacles to achieving the biomimetic equivalent of the inspiring enzyme catalysed cyclisation of squalene 2,3-epoxide (**2.12**) leading to lanosterol (**2.13**), the biological precursor to the far more common steroid skeleton (Scheme 2.6). An enormous amount of effort has been put into the understanding of this polycyclisation and to the development of a variety of ingenious (though not usually biogenetically-inspired) methods to override the intrinsic reactivity of the natural precursors and allow formation of the 6-membered C-ring necessary for the steroid skeleton, *via* related polyene cyclisations.<sup>60,72,80</sup>





Scheme 2.6

Another example of adverse intrinsic reactivity is the attempted biomimetic construction of the morphine skeleton (salutaridine, **2.15**) via the *para-ortho* oxidative coupling of the key biological precursor reticuline (**2.14**, Scheme 2.7). Although this coupling is readily achieved in nature,<sup>81</sup> numerous attempts to mimic it chemically have produced mixtures of the *ortho-para* ( $\leq 53\%$ ) and the *para-para* products ( $\leq 4\%$ ) with, at most, traces ( $\leq 0.03\%$ ) of the required *para-ortho* product.<sup>64</sup> A subsequent report of a low yield of the *para-ortho* coupled product using modified substrates<sup>82</sup> does not alter the perception that the inherent regioselectivity for this type of coupling reaction is unfavourable.

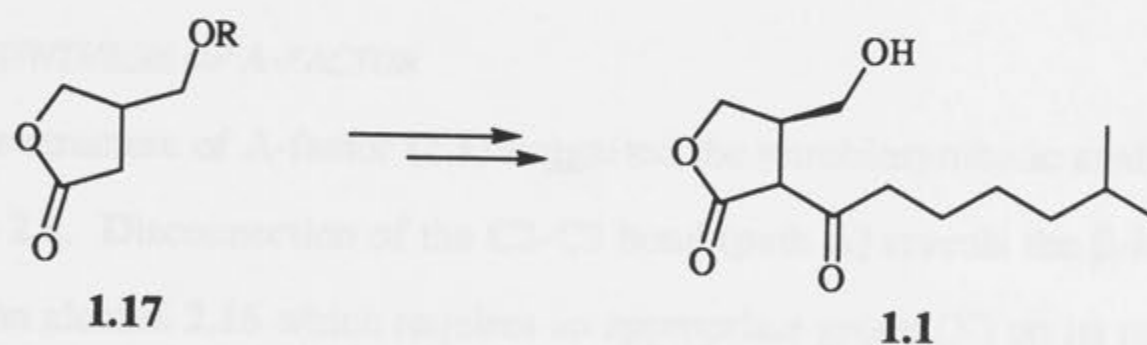


Scheme 2.7 Attempted Biomimetic Syntheses of the Morphine Ring System

Today, biosynthetic considerations are often routinely incorporated into the retrosynthetic planning of the synthesis of natural products. Unfortunately, it remains difficult to predict which putative biological precursors will have favourable intrinsic chemical reactivity and a 'trial and error' approach is often required.

## 2.2 A BIOMIMETIC APPROACH TO THE SYNTHESIS OF A-FACTOR AND RELATED BUTANOLIDE AUTOREGULATORS

Previous syntheses of A-Factor (**1.1**) (see Section 1.6.6) all follow routes determined by chemical expedience rather than by biosynthetic considerations.<sup>18,20,29,51,52</sup> All are based on the C2-acylation of a protected 3-hydroxymethyl butyrolactone (**1.17**) which itself was synthesised by a variety of non-biomimetic routes. The acylation of the butyrolactone **1.17** is very unlikely to be biomimetic; it has not been proposed as such and there exists no evidence of this synthetic intermediate existing in nature.



In contrast to the traditional approach of these previous syntheses, we were led to consider the viability of developing an improved route to this remarkable bioregulator based on our proposal for its biosynthesis which is described in the following section.



### 2.2.1 Proposed Biosynthesis of A-Factor

At the inception of this project very limited evidence existed as to the biological origin of A-factor (1.1). However genetic studies had been performed on the genetic determinant of A-factor biosynthesis.

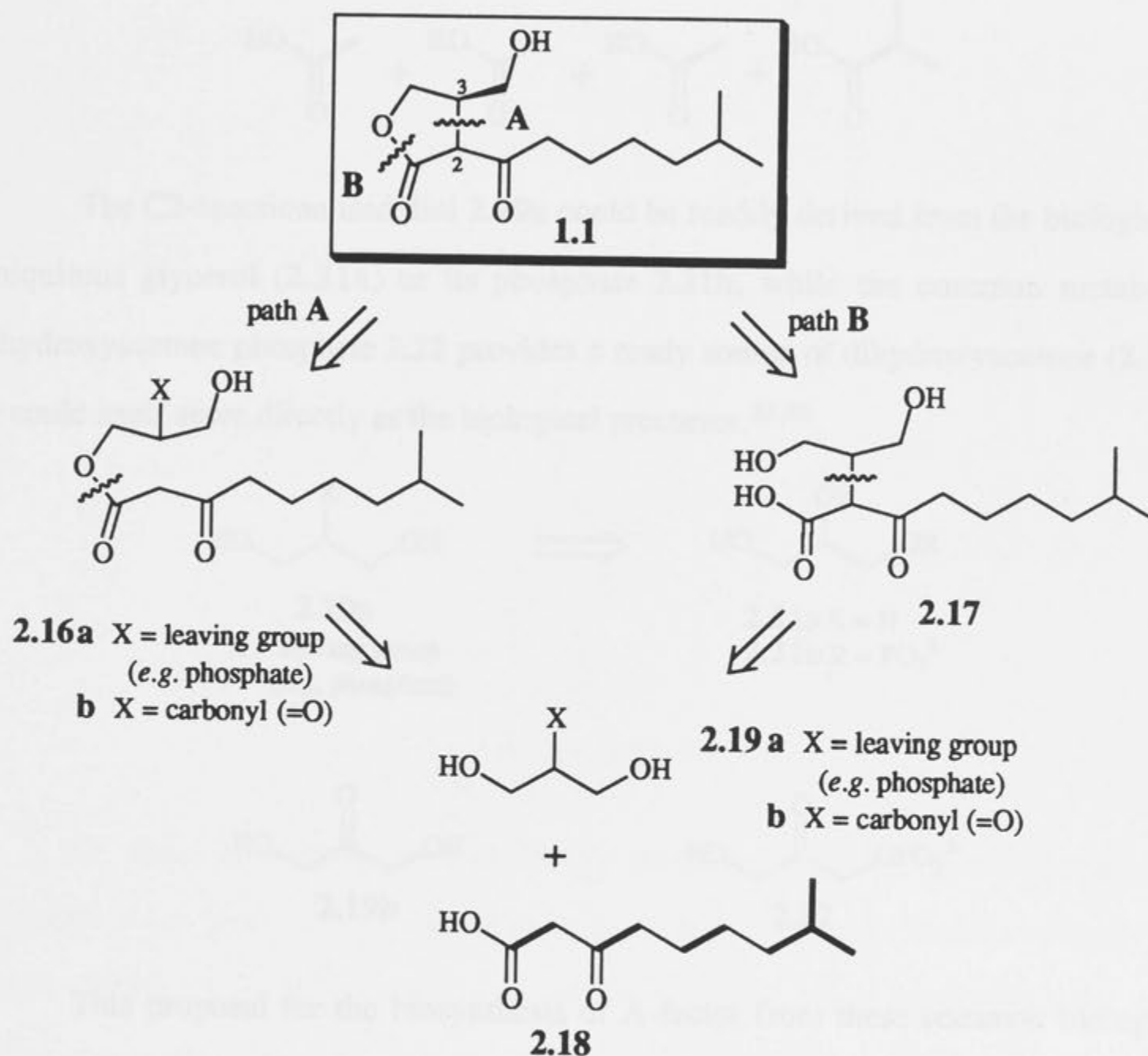
In 1984, Horinouchi and coworkers identified and cloned a fragment of extrachromosomal DNA from *S. bikiniensis* ('*afs A*' gene) which appeared wholly responsible for the ability to produce A-factor.<sup>11</sup> It was suggested that the location of this gene on an "*unstable extrachromosomal element, possibly a plasmid*", (where it could be easily damaged by a variety of mutagenic agents) explained the ease with which A-factor deficient mutants could be produced.<sup>14</sup> They developed (and patented<sup>83</sup>) a method for the overproduction of A-factor in normally non-producing strains of *Streptomyces* by the insertion of a plasmid incorporating multiple copies of the excised gene.<sup>84</sup> The small size of the DNA fragment (1100 base pairs) led to their proposal that it coded for only a single enzyme which facilitated the synthesis of A-factor from common metabolites of *Streptomyces* species.

#### RETROBIOSYNTHESIS OF A-FACTOR

The structure of A-factor (1.1) suggested the retrobiosynthetic analysis outlined in Scheme 2.8. Disconnection of the C2-C3 bond (path A) reveals the  $\beta$ -ketoester of a three carbon alcohol 2.16 which requires an appropriate group (X) on its central carbon to provide activation towards cyclisation. It was envisaged that this activation may be achieved with either a biological leaving group (2.16a, *e.g.* X = phosphate), in which case the cyclisation would involve intramolecular alkylation, or by a carbonyl group (2.16b) which allows cyclisation *via* an intramolecular Knoevenagel condensation. The esters 2.16a or 2.16b could be further disconnected to the  $\beta$ -ketoacid 2.18 and the C2 functionalised 1,3-propanediols 2.19a or 2.19b, respectively.

Alternatively, the lactone moiety could be cleaved first (path B) to generate the  $\alpha$ -alkyl- $\beta$ -ketoacid intermediate 2.17 which could be derived from the same precursors

as for path A (2.18 and 2.19a or 2.19b) *via* intermolecular alkylation or Knoevenagel condensation. This route is less attractive than path A, since the intermolecular C-C bond formation involved would be expected to be a higher energy process than the intramolecular equivalent in path A.



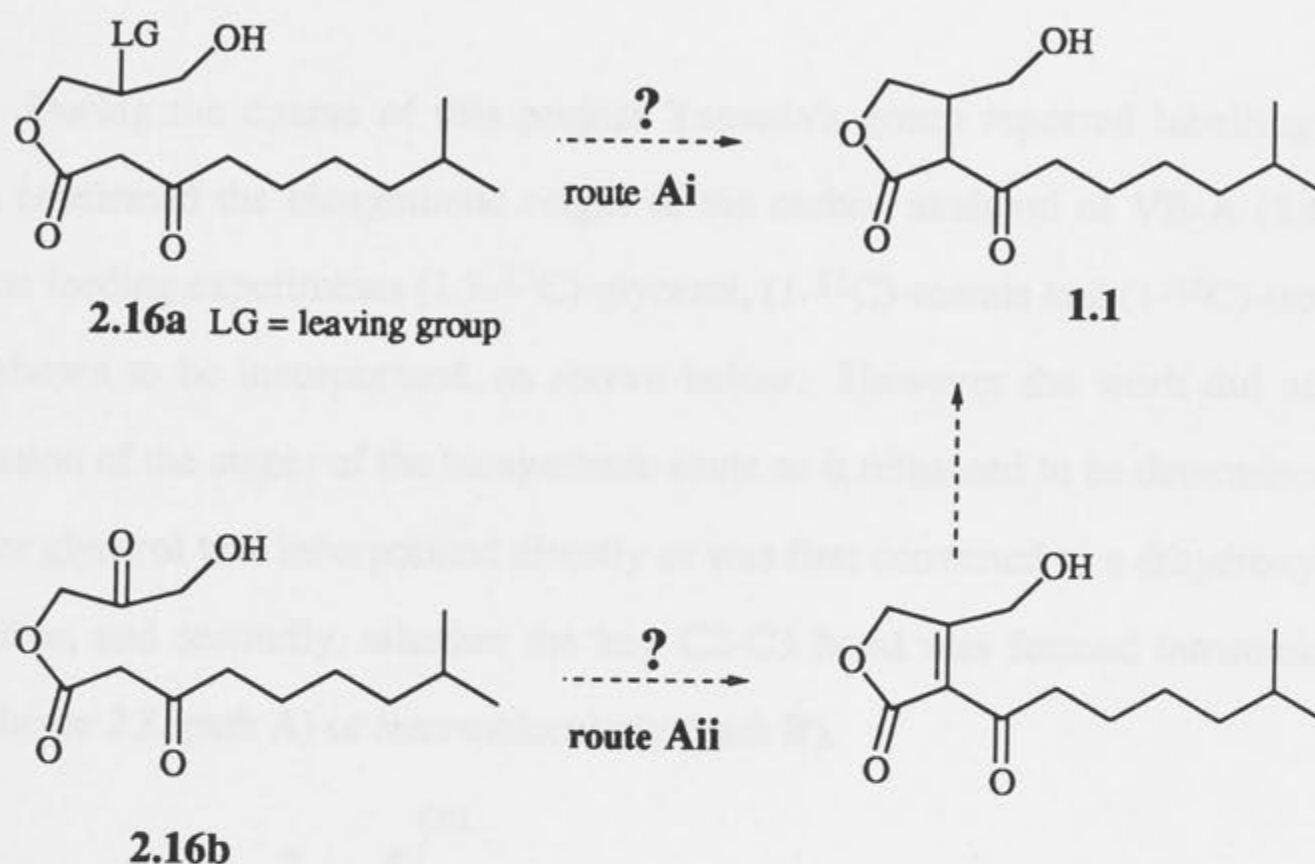
**Scheme 2.8** Retrobiosynthetic Analysis of A-Factor

It was recognised that the carboxylic acid 2.18 would be likely to exist as an enzyme-bound thioester 2.20 which would be readily available to the organism *via* normal fatty acid biosynthesis using an isobutyrate starter unit followed by three acetate units. It appeared likely that the variety of alkyl chains observed in the other butanolide autoregulators (1.2-1.10) result from the incorporation of different numbers of acetate units and/or other starter units into such a thioester.





*intermolecular* processes required for the C2-C3 bond formation using the alternative biochemical pathway **B** (see Scheme 2.8). Thus formation of the required  $\gamma$ -lactone moiety *via* the cyclisation of the  $\beta$ -ketoester intermediates **2.16a** or **2.16b**, as depicted in Scheme 2.9, was chosen for initial investigation.



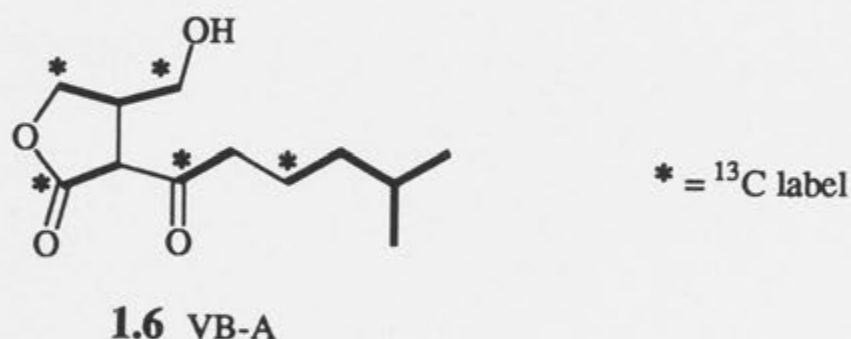
Scheme 2.9

Of these two approaches to the synthesis of A-factor, the intramolecular alkylation route (route Ai) seemed initially more attractive than the Knoevenagel-type process (route Aii) as it was more direct and had clear potential for development as an asymmetric route. The following chapter describes our investigations into the viability of this method of  $\gamma$ -lactone formation, using model chemical systems.



### 2.3 POSTSCRIPT: CONFIRMATION OF THE POSTULATED BIOSYNTHESIS OF BUTANOLIDE AUTOREGULATORS

During the course of this project Yamada's group reported labelling studies which confirmed the biosynthetic origin of the carbon skeleton of VB-A (**1.6**).<sup>87</sup> In separate feeding experiments (1,3-<sup>13</sup>C)-glycerol, (1-<sup>13</sup>C)-acetate and (1-<sup>13</sup>C)-isovalerate were shown to be incorporated, as shown below. However the work did not allow elucidation of the stages of the biosynthetic route so it remained to be determined firstly whether glycerol was incorporated directly or was first converted to a dihydroxyacetone derivative, and secondly, whether the key C2-C3 bond was formed *intramolecularly* (cf. Scheme 2.8, path A) or *intermolecularly* (path B).



Whilst it was recognised that our biomimetic studies could not prove which of these routes was utilised in nature, a large difference in facility may suggest which was more probable, as well as providing an attractive alternative synthesis.

## CHAPTER THREE

### SYNTHETIC APPROACHES TO BUTANOLIDES VIA INTRAMOLECULAR ALKYLATION

*If at first you don't succeed.....*

*Try once more.....and then give up.*

Coffee cup (RSC tea room 1992)

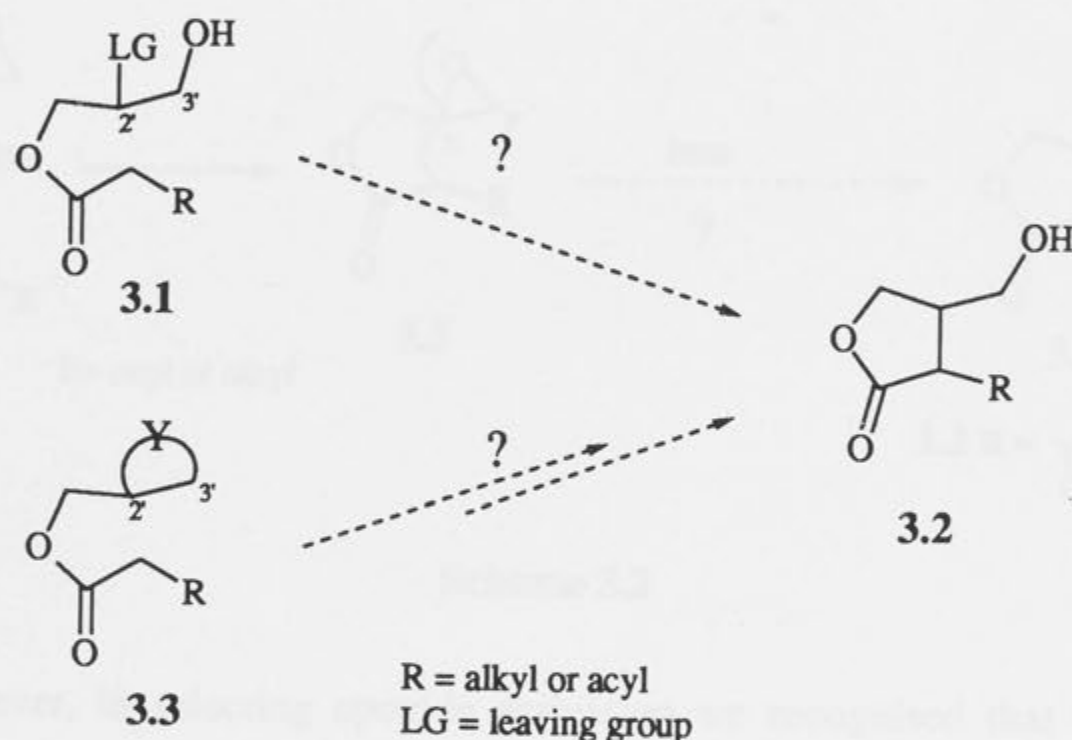


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### 3.1 BUTANOLIDES VIA INTRAMOLECULAR ALKYLATION?

3'-Hydroxypropyl esters **3.1** incorporating a leaving group (LG) at C2' could potentially form the 3-hydroxymethyl butanolides **3.2** required for the synthesis of A-factor (**1.1**) upon intramolecular alkylation. However, the use of these esters as model substrates is complicated by the need for protection of the primary hydroxyl at C3' to prevent unwanted reactivity under basic conditions.



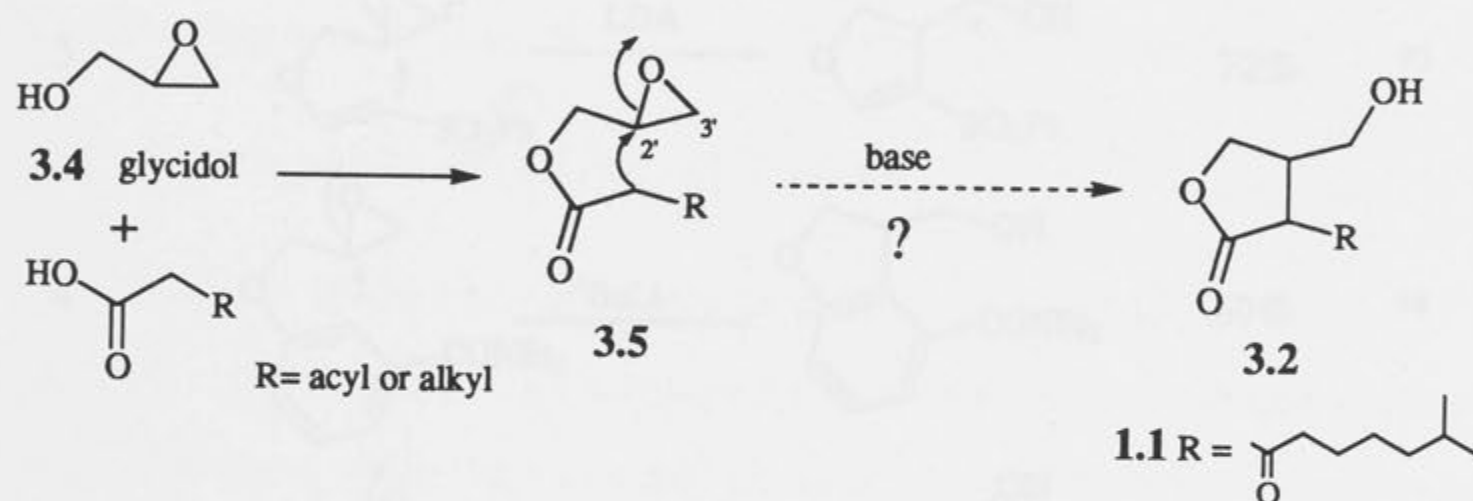
Scheme 3.1

An attractive alternative approach is to tether the C2' and C3' positions of ester **3.1** with a group Y to form a ring, as in **3.3**, which incorporates protection of the hydroxyl whilst maintaining C2' activation. Clearly, the ring must cleave easily and be able to form the required hydroxymethyl substituent after opening. The simplest suitable ring is an epoxide (**3.3**, Y=O), although cyclic sulfites, sulfates<sup>88</sup> or phosphates are other possibilities. The ready commercial availability of 2,3-epoxy-1-propanol (glycidol, **3.4**)<sup>89</sup> made the epoxide system the most convenient for initial investigations.



### 3.1.1 The Epoxide Ring as an Electrophile

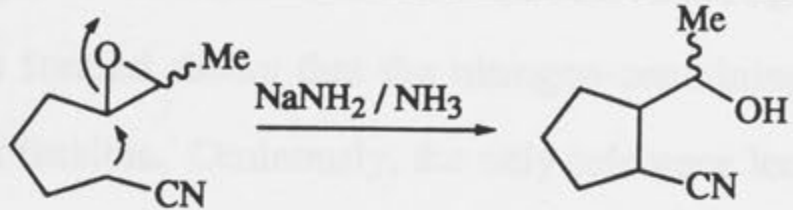
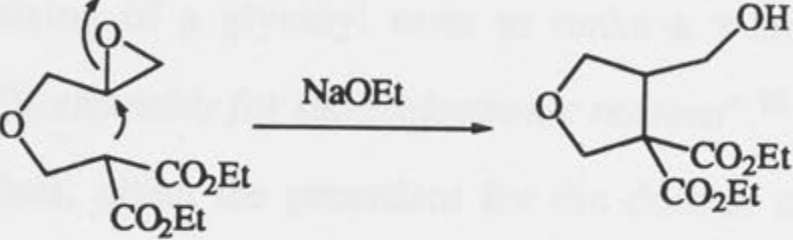
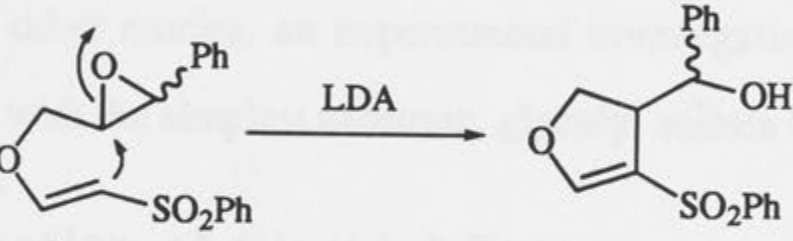
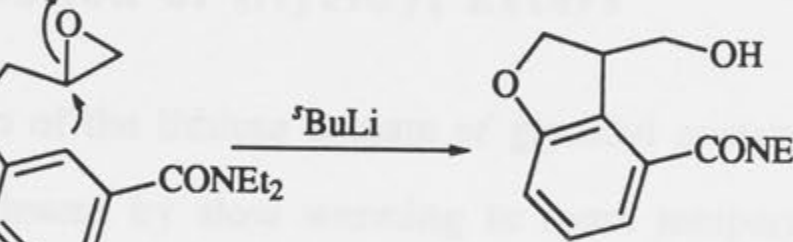
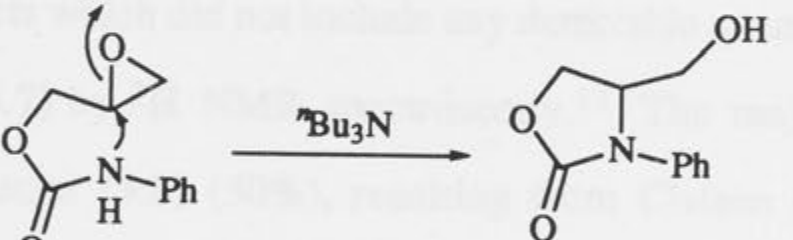
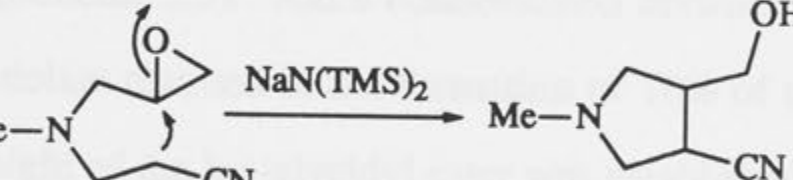
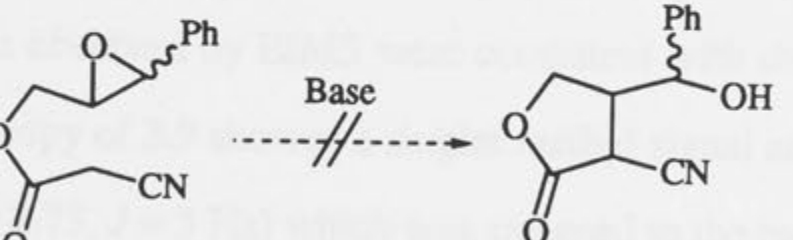
Glycidyl esters are readily prepared by esterification of the relevant acid with inexpensive and readily available glycidol (3.4). Optically active forms of glycidol are also commercially available, allowing easy entry to a possible asymmetric synthesis. With an acyclic leaving group at C2', access to optically pure starting materials is considerably more difficult.<sup>90</sup> By incorporation of the necessary acyl substituent in the ester (3.5, R = CO(CH<sub>2</sub>)<sub>4</sub>CH(CH<sub>3</sub>)<sub>2</sub>) this cyclisation route seemed to offer the potential for a remarkably efficient route to A-factor (1.1).



Scheme 3.2

However, in selecting epoxide activation we recognised that we were also introducing another possible site for attack, the terminal epoxide carbon C3' on 3.5. Since the C3' position is less substituted than C2' it offers a potentially significant alternative site for enolate attack. In order to assess the feasibility of achieving regioselective C2' attack, a literature search was undertaken for examples of 5-membered ring formation *via* the intramolecular opening of epoxides. Table 3.1 contains selected examples of the intramolecular attack on epoxides at the required secondary site by a variety of stabilised anions.

**Table 3.1 Literature Examples of 5-Membered Ring Formation via the Intramolecular Opening of Epoxides**

Entry No.	Reaction	Yield	Ref.
1		77%	91
2		"high"	92
3		72%	93
4		67%	94
5		92%	95
6		67%	96
7		0%	93

Entries 2, 4, 5 and 6 (Table 3.1) show that 5-membered rings can be formed in good yields *via* epoxide opening at the required epoxide carbon, even when the alternative epoxide site remains unsubstituted and thus might be expected to be more reactive. In no case was a 6-membered ring product reported. It appears that the kinetic advantage of forming 5 rather than 6 membered rings<sup>97</sup> outweighs the advantage of



attack at a less sterically hindered primary carbon. Entry 2 (Table 3.1) provides precedent for the use of enolate stabilised anions in this sort of cyclisation. No precedent for  $\gamma$ -lactone formation was found, although Entry 5 in which an oxazolidinone is formed shows that the nitrogen-containing analogue of our desired transformation is feasible. Ominously, the only reference located which mentioned the attempted cyclisation of a glycidyl ester to make a  $\gamma$ -lactone (Entry 7, Table 3.1) reported failure, "*presumably for stereoelectronic reasons*".<sup>93</sup>

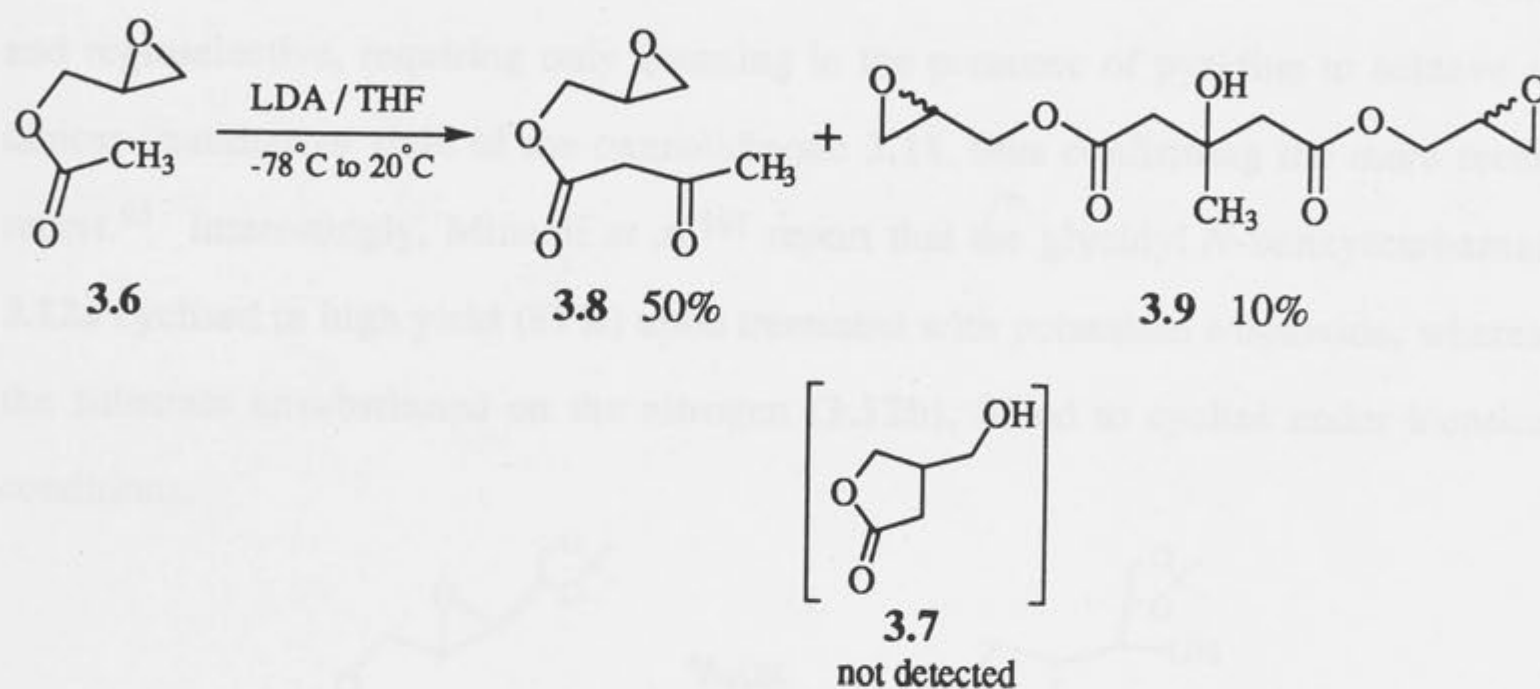
Nevertheless, given the precedent for the desired mode of epoxide opening provided by the other entries, an experimental investigation of the cyclisation was initiated, starting with the simplest substrate, glycidyl acetate (3.6).

### 3.1.2 Cyclisation of Glycidyl Esters

Formation of the lithium enolate of glycidyl acetate (3.6) at  $-78^{\circ}\text{C}$  (1.1 eq., LDA, THF), followed by slow warming to room temperature yielded a complex mixture of products which did not include any detectable quantities of 3-hydroxymethyl butyrolactone (3.7) by  $^1\text{H}$  NMR spectroscopy.<sup>18</sup> The major product isolated was glycidyl acetoacetate (3.8) (50%), resulting from Claisen self-condensation of the starting material (Scheme 3.3). Aldol condensation between this product and another molecule of the enolate resulted in the formation of 10% of the bis-glycidyl ester 3.9. The molecular weight of the bis-glycidyl ester was established by CIMS to be 274 and the fragmentations observed by EIMS were consistent with the proposed structure 3.9.  $^1\text{H}$  NMR spectroscopy of 3.9 showed a singlet methyl signal at  $\delta$  1.39 and a four proton pseudo-doublet ( $\delta$  2.73,  $J = 5$  Hz) which was assigned to the two methylene groups  $\alpha$  to the ester carbonyls. A system of resonances characteristic of a glycidyl ester moiety was shown to represent two such groups by its ten proton integration measurement. This structure has interesting diastereomeric possibilities since the central carbon is pseudoasymmetric, *i.e.* it is only chiral when the two epoxides differ in configuration. Thus, two *meso* forms of this molecule are expected in addition to a *dl*-pair. However, the three forms are evidently very similar by  $^1\text{H}$  NMR, as would be expected for a



molecule with such widely separated asymmetric centres, since the only indication of additional sets of peaks was some slight line broadening. A further 12% of the mass of the starting material was isolated as an unidentified mixture of compounds which appeared, by  $^1\text{H}$  NMR, to contain intact glycidyl moieties as indicated by complex resonances near  $\delta$  4.5, 4.0, 3.2, 2.9 and 2.7. Similar mixtures of self-condensation products were obtained using a more dilute reaction mixture, a lower reaction temperature ( $-60^\circ\text{C}$ ) or a Lewis acid ( $\text{BF}_3\cdot\text{OEt}_2$ ).

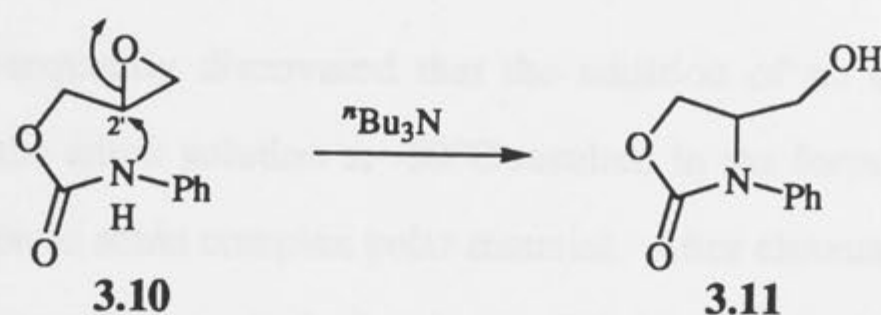


Scheme 3.3

Self-condensation is a commonly observed side reaction of ester enolates unless the ester carbonyl is sterically crowded (*e.g.* *t*-butyl esters) or the anion has additional resonance stabilisation.<sup>98</sup> However, in this case the tendency to self-condense was expected to be minimised by the use of the hindered lithium amide base (LDA) and low reaction temperatures.<sup>99</sup> It appears that intramolecular attack on the epoxide is not facile enough to offer an alternative reaction path.

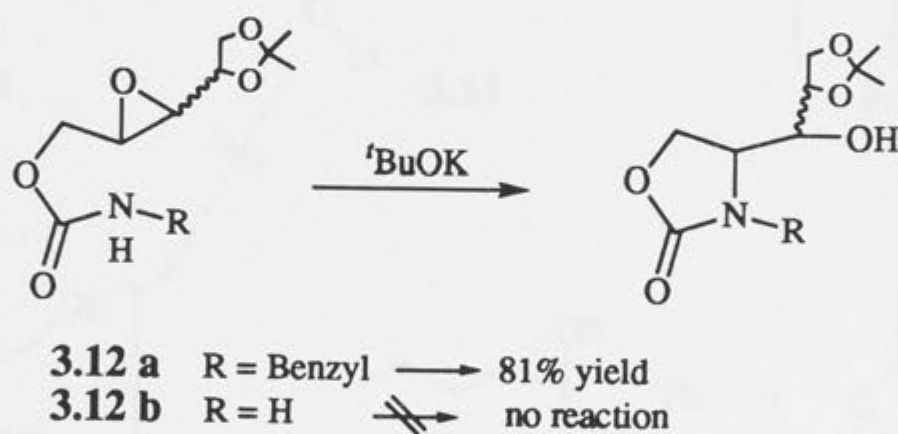
This prompted us to re-examine the reported efficient intramolecular epoxide opening of glycidyl phenyl carbamate<sup>95</sup> (**3.10**) as listed in Table 3.1 (Entry 5). The paper describes exclusive C2' attack by nitrogen under mildly basic conditions to give the oxazolidinone **3.11** (Scheme 3.4); this is a nitrogen-containing analogue of our desired reaction. Since an earlier report<sup>100</sup> described cyclisation through C3' we thought it prudent to repeat the reaction.





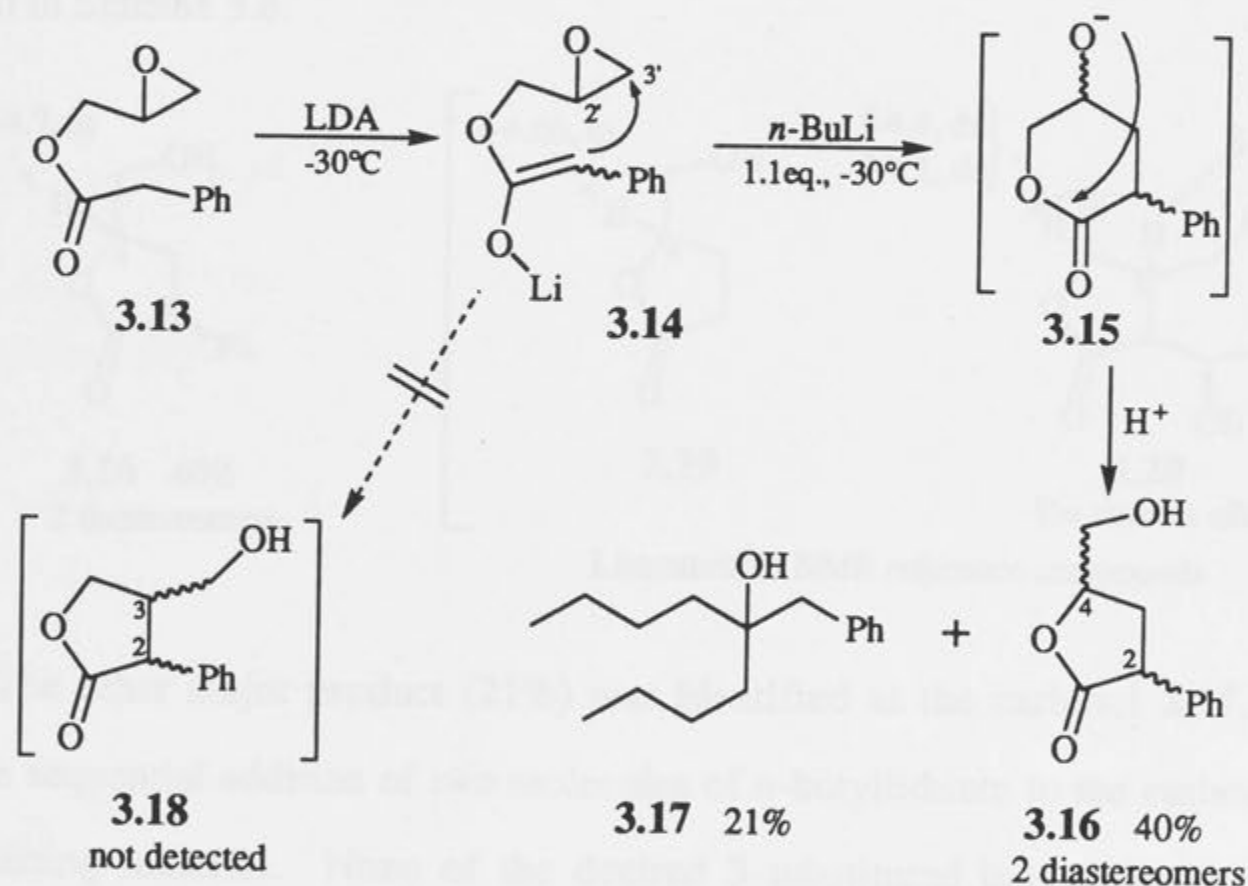
Scheme 3.4

Glycidyl phenyl carbamate (3.10) was prepared from glycidol and phenyl isocyanate by the method of Farrissey *et al.*<sup>95</sup> Cyclisation was indeed found to be facile and regioselective, requiring only warming in the presence of pyridine to achieve an almost quantitative yield of the oxazolidinone 3.11, thus confirming the more recent report.<sup>95</sup> Interestingly, Minami *et al.*<sup>101</sup> report that the glycidyl *N*-benzylcarbamate 3.12a cyclised in high yield (81%) upon treatment with potassium *t*-butoxide, whereas the substrate unsubstituted on the nitrogen (3.12b), failed to cyclise under identical conditions.

Scheme 3.5 Related Literature Reactions by Minami *et al.*

To examine the possibility that the presence of nitrogen substituents in 3.10 and 3.12a was promoting cyclisation, possibly *via* steric or electronic effects, we prepared glycidyl phenylacetate (3.13),<sup>102</sup> the direct carbon analogue of glycidyl phenylcarbamate (3.10). The lithium enolate of glycidyl phenylacetate (3.14) was formed using LDA. The first characteristic noted was its surprising stability, since virtually no reaction took place even after 22 hours at room temperature. More than 75% of unreacted starting material was recovered after this period. Complete anion formation was confirmed by quenching a sample of reaction mixture with an excess of methyl iodide; NMR and GC analysis revealed > 95% methylated starting material.

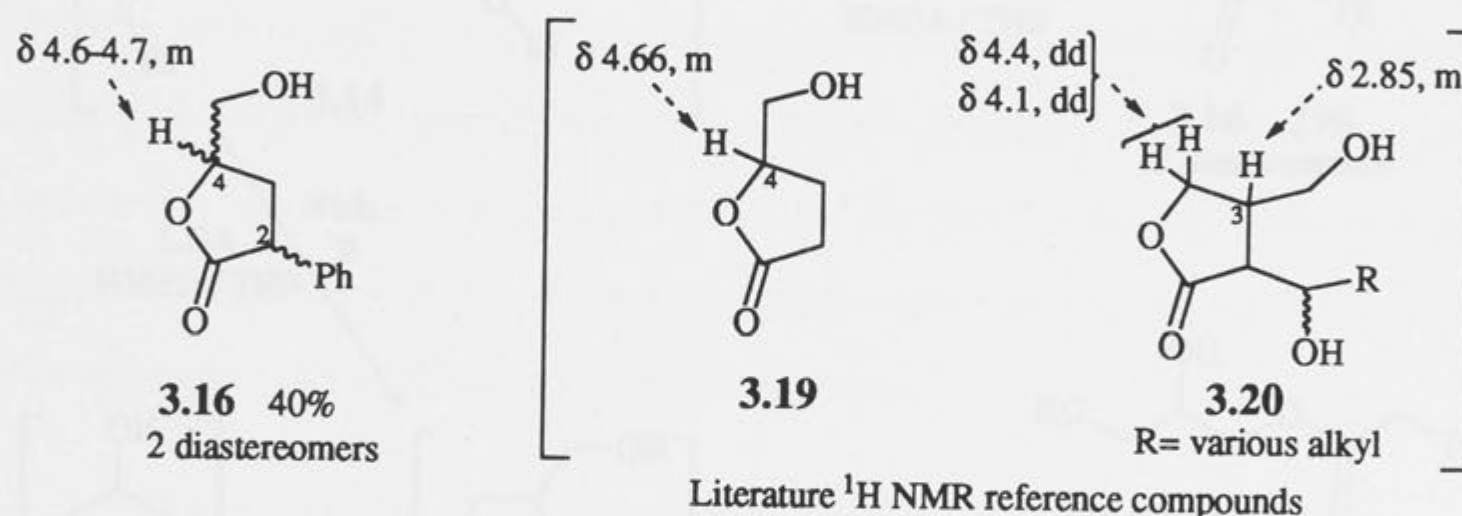
It was subsequently discovered that the addition of an extra equivalent of *n*-butyllithium to the anion solution at  $-30^{\circ}\text{C}$  resulted in the formation of two major products, in addition to some complex polar material. After chromatography, the more polar of the major products was isolated as colourless needles in 40% yield. Its  $^1\text{H}$  NMR spectrum lacked both the benzylic methylene singlet ( $\delta$  3.6) and the three epoxide resonances ( $\delta$  3.2, 2.9 and 2.7) of the starting material, suggesting that cyclisation had occurred. In addition, the infra-red carbonyl stretch absorption at  $1771\text{ cm}^{-1}$  suggested that a  $\gamma$ -lactone had been formed.<sup>103</sup> A molecular ion at  $m/z$  192 in the EIMS was consistent with either the desired 3-hydroxymethyl substituted butyrolactone **3.18** or the 4-hydroxymethyl alternative **3.16** (Scheme 3.6).



Scheme 3.6



Comparison of the  $^1\text{H}$  NMR data of the product with those reported for reference  $\gamma$ -lactones **3.19**<sup>104</sup> and **3.20**<sup>25</sup> offered a distinction between these two possible structures (see below). The observed downfield methine multiplet at  $\delta$  4.6-4.7 is expected for the 4-hydroxymethyl compound (*cf.* **3.19**) but not for 3-hydroxymethyl butyrolactones (*e.g.* **3.20**). In addition, the characteristic pair of doublets of doublets at  $\delta$  4.4 and  $\delta$  4.1 due to the C4 methylene protons of the 3-hydroxymethyl butyrolactones **3.20** was absent. The remaining  $^1\text{H}$  NMR data as well as the  $^{13}\text{C}$  spectrum supported the assignment of the product as a diastereomeric mixture of the 4-hydroxymethyl butyrolactones **3.16**. These probably are formed by initial enolate carbon attack at C3' of **3.14** giving the  $\delta$ -lactone intermediate **3.15**, followed by translactonisation, as depicted in Scheme 3.6.

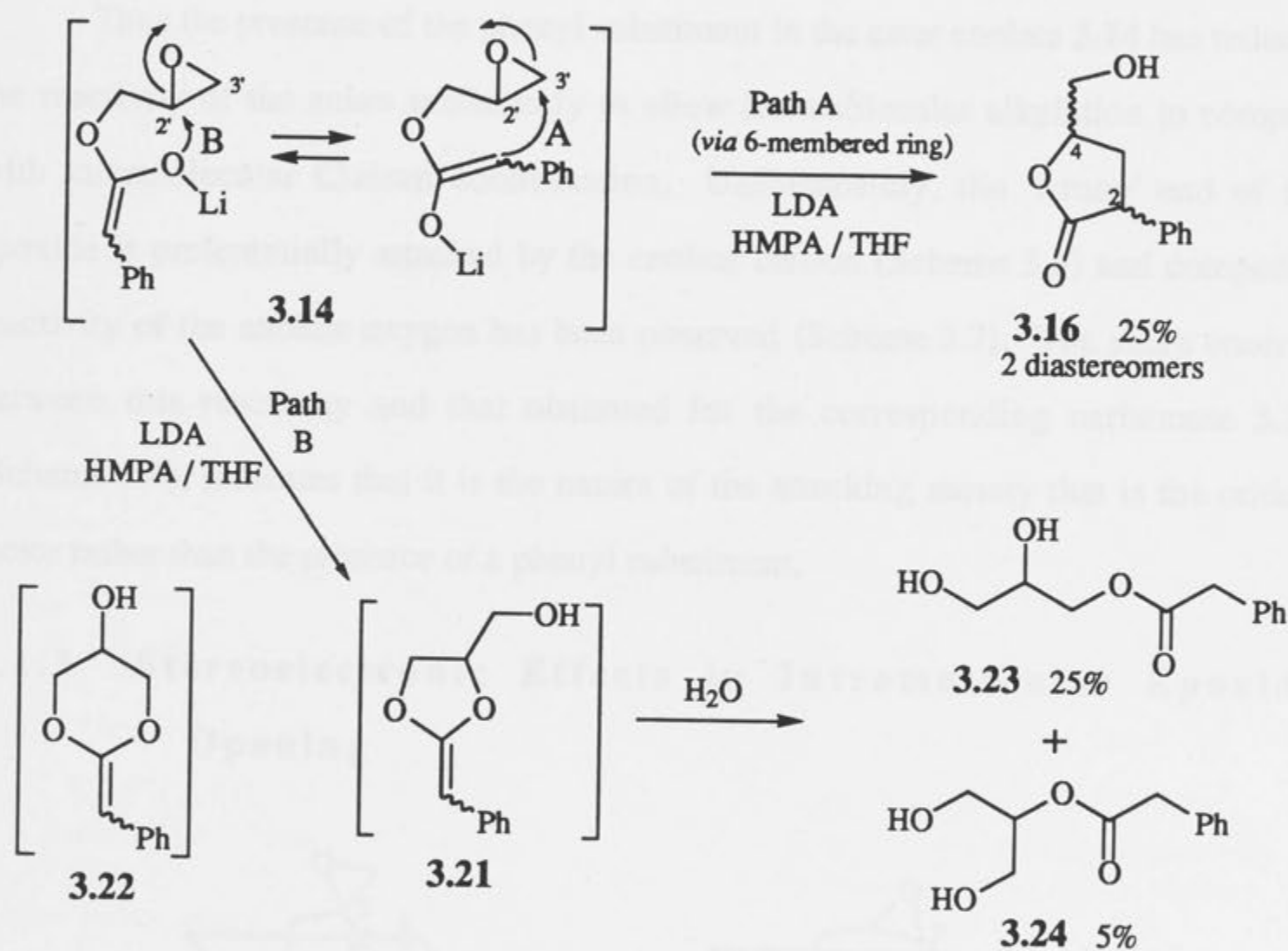


The other major product (21%) was identified as the carbinol **3.17**, resulting from the sequential addition of two molecules of *n*-butyllithium to the carbonyl of the ester starting material. None of the desired 3-substituted butyrolactone **3.18** was detected by  $^1\text{H}$  NMR in any chromatographic fraction.

The additional *n*-butyllithium is believed to enhance enolate reactivity by deprotonating the diisopropyl amine in the reaction mixture, thus breaking up the H-bonded complex between the lithium enolate and the amine in solution and leaving the enolate free to react. This complex was postulated by Seebach's group<sup>105</sup> to explain the incomplete (*ca* 30%) deuteration of lithium enolate anions. The addition of one equivalent of *n*-butyllithium increases deuteration to *ca* 95%. Unfortunately, the use of this highly nucleophilic base also causes extensive side reactions.



Hexamethylphosphoramide (HMPA) is often used as an alternative means of enhancing enolate reactivity.<sup>106</sup> For the cyclisation of anion **3.14**, the inclusion of 10% HMPA in the THF solvent resulted in the formation of 25% of the previously isolated lactone **3.16** (Path A, Scheme 3.7). In addition, 30% of an inseparable mixture of the glycerol esters **3.23** and **3.24** (5:1 ratio) was isolated. These diols were identified by comparison of their <sup>1</sup>H NMR spectra with other known 1- and 2-monoglycerides<sup>107</sup> Overall recovery was low due to extensive polar by-product formation. Again, no 4-hydroxymethyl butyrolactone **3.18** was detected.



Scheme 3.7

It is unlikely that the diols **3.23** and **3.24** resulted from hydrolysis of the starting epoxide **3.13**, since the reaction was performed under anhydrous conditions and the reaction mixture was buffered to pH 6.5 during aqueous work-up. In addition, a subsequent acyl migration would be required to form the observed 2-acyl glycerol **3.24**. It is much more likely that the diols arose *via* enolate oxygen attack on the secondary epoxide site (C2', Scheme 3.7, Path B) producing the unstable ketene acetal intermediate **3.21**. Hydrolysis of this acetal could give the two observed

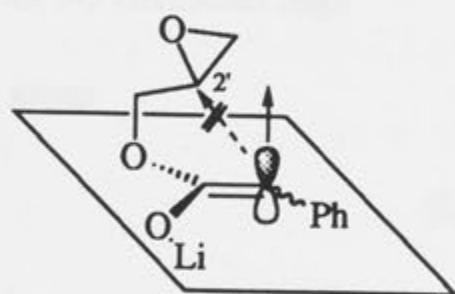
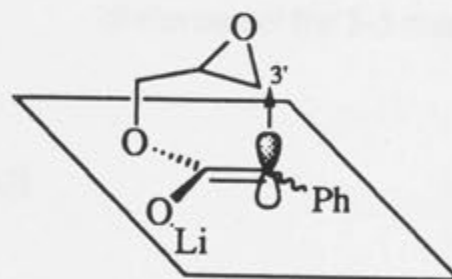


monoglycerides **3.23** and **3.24**. Oxygen attack on C3' (giving **3.22**) is less likely given the kinetic advantages of 5 vs. 6 membered ring formation in cyclisations of this type.<sup>97</sup> Note that **3.22** cannot readily form the observed 2-acyl glycerol **3.24** *via* hydrolysis.

Careful re-examination of the  $^1\text{H}$  NMR spectra of polar fractions from the cyclisation without HMPA (Scheme 3.6), revealed *ca* 5% of the diol **3.23** and a trace of diol **3.24**. The observed formation of a greater proportion of these diols in the presence of HMPA is consistent with the recognised ability of polar aprotic solvents to promote O-alkylation of ambident enolate anions.<sup>106</sup>

Thus the presence of the phenyl substituent in the ester enolate **3.14** has reduced the reactivity of the anion sufficiently to allow intramolecular alkylation to compete with intermolecular Claisen condensation. Unfortunately, the 'wrong' end of the epoxide is preferentially attacked by the enolate carbon (Scheme 3.6) and competing reactivity of the enolate oxygen has been observed (Scheme 3.7). The sharp contrast between this reactivity and that observed for the corresponding carbamate **3.10** (Scheme 3.4), indicates that it is the nature of the attacking moiety that is the critical factor rather than the presence of a phenyl substituent.

### 3.1.3 Stereoelectronic Effects in Intramolecular Epoxide Opening

**3.14a****3.14b**

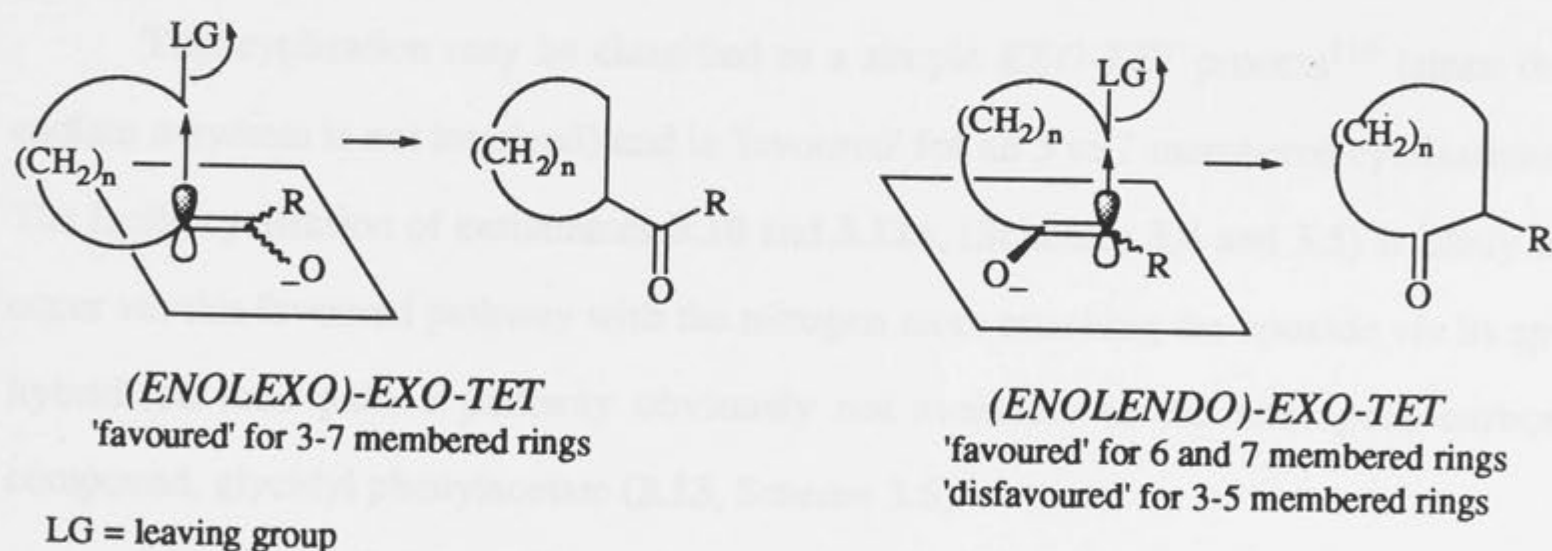
For the opening of an epoxide by an enolate carbanion to proceed, the  $\pi$ -orbitals of the nucleophilic enolate carbon must achieve an attack trajectory which is co-linear with the epoxide C-O bond at the attack site to maximise orbital overlap.<sup>108</sup> Studies with Dreiding models show that for an intramolecular process, the minimum chain length connecting the reacting centres for which this co-linearity is easily achieved is



six atoms (e.g. 3.14b). When a 5-atom chain is used (e.g. 3.14a) considerable angle strain appears to be involved in achieving the required transition state geometry.

Baldwin and Lusch<sup>109</sup> have made a systematic study of such stereoelectronic effects involved in various enolate cyclisations, as an extension of Baldwin's well-known previous study of general cyclisation reactions.<sup>110</sup> They concluded that it is of critical importance whether the enolate C=C bond is *ENDO* (i.e. inside) or *EXO* (i.e. outside) to the ring being formed (see Scheme 3.8).

*"Especially when the enolate C=C bond is ENDO to the ring being formed in the alkylation, the planarity of the enolate system curtails the freedom of movement of the chain of the leaving group and approach of the electrophilic halide [or in our case, epoxide] carbon on a trajectory perpendicular to the enolate plane at the  $\alpha$ -carbon atom, is an important factor in determining the ability of the reactive termini to meet in proper orientation."*<sup>109</sup>



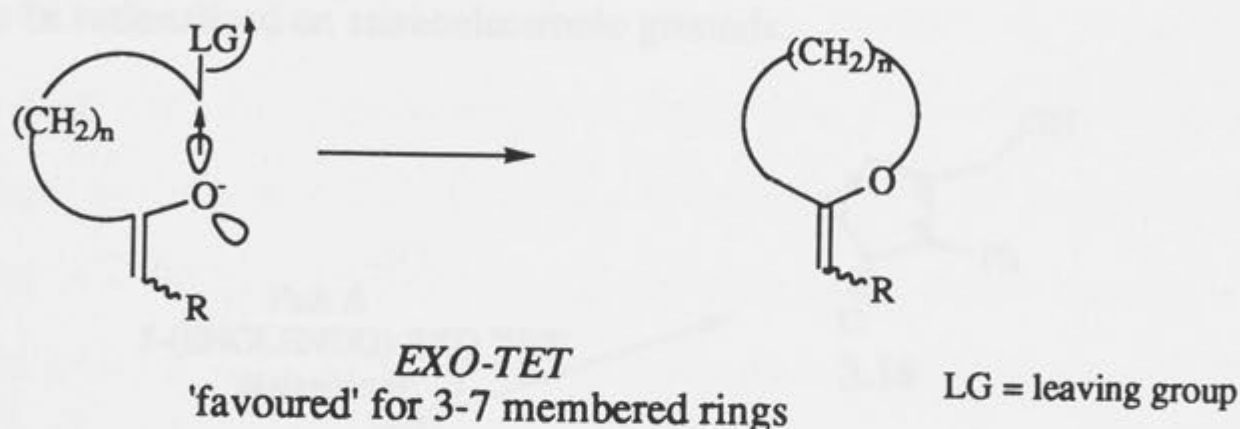
Scheme 3.8

They propose that when the chain of atoms connecting and including the reacting sites is 6 or more atoms long there is sufficient flexibility to achieve the required geometry for cyclisation even when the enolate C=C bond is *ENDO*, i.e. 6-(ENOLEXO)-EXO-TET and 6-(ENOENDO)-EXO-TET alkylations are 'favoured' processes (see Scheme 3.8). However, for 3 to 5 membered cyclisations, the required reaction geometry can only be easily achieved when the enolate C=C bond is *EXO*. The flexibility restrictions caused when the enolate C=C is *ENDO* makes the required bond



overlap difficult to achieve; *i.e.* 3 to 5-(*ENOLENDO*)-*EXO-TET* cyclisations are 'disfavoured' (see Scheme 3.8).

When intramolecular enolate alkylation occurs *via* attack by the enolate oxygen, the necessary reaction geometry is much easier to achieve. The ability of the enolate oxygen to react *via* its  $sp^2$  hybridised lone pairs means that it may attack *within* the enolate plane, a process which is much less stereoelectronically demanding than attack by  $\pi$ -orbitals perpendicular to the enolate plane (Scheme 3.9).<sup>111</sup>



Scheme 3.9

This cyclisation may be classified as a simple *EXO-TET* process<sup>110</sup> (since the enolate  $\pi$ -system is not involved) and is 'favoured' for all 3 to 7 membered cyclisations. The facile cyclisation of carbamates **3.10** and **3.12a**, (Schemes 3.4 and 3.5) is likely to occur *via* this favoured pathway with the nitrogen atom attacking the epoxide *via* its  $sp^2$  hybridised lone pair, a pathway obviously not available to the analogous carbon compound, glycidyl phenylacetate (**3.13**, Scheme 3.6).

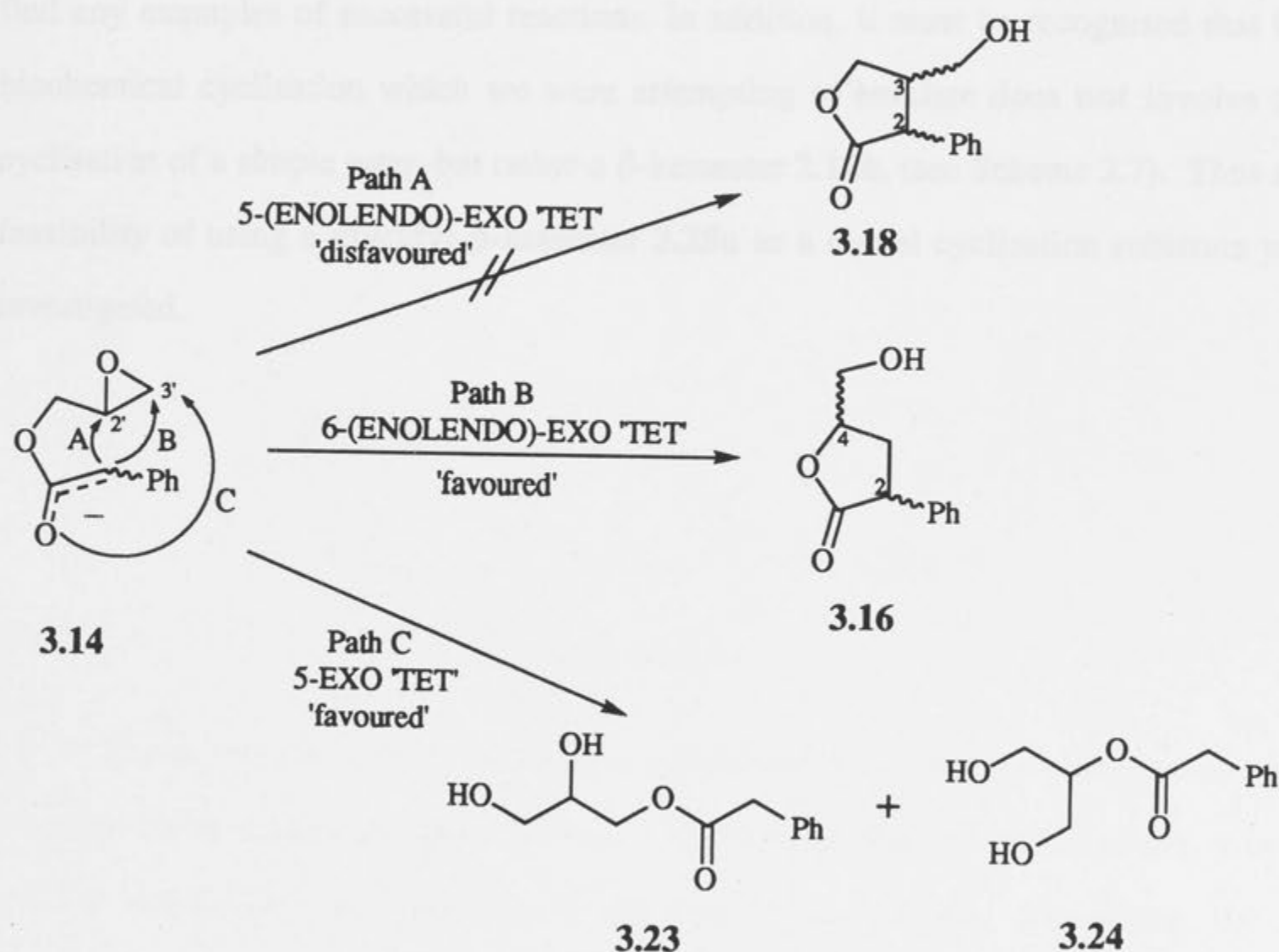
The reason for the failure of the N-unsubstituted carbamate **3.12b** to cyclise under conditions which readily cyclised the analogous N-benzyl carbamate **3.12a** (see Scheme 3.5) is not clear since the relative contribution of differences in acidity, nucleophilicity and stereoelectronic effects in the two substrates are difficult to assess.

The results observed for the cyclisation of the glycidyl phenylacetate anion **3.14** (Scheme 3.11) are in accordance with Baldwin's analysis of such types of cyclisations. Baldwin and Lusch<sup>109</sup> consider the opening of 3-membered rings as being stereoelectronically intermediate between a normal tetrahedral and trigonal system. Since the rules for *EXO-TET* and *EXO-TRIG* cyclisations are the same, we will denote

epoxide openings as *EXO-TET* processes for simplicity. Thus, the required enolate carbon attack at C2' (Path A, Scheme 3.10) can be considered an example of a 'disfavoured' 5-(*ENOLENDO*)-*EXO-TET* process in competition with two alternative 'favoured' pathways:

- enolate carbon attack at C3' (Path B)- a 6-(*ENOLENDO*)-*EXO-TET* process;
- enolate oxygen attack at C2' (Path C)- a 5-*EXO-TET* process.

The failure of the cyclisation reaction to generate the desired  $\gamma$ -lactone may therefore be rationalised on stereoelectronic grounds.



Scheme 3.10

The previously discussed literature examples of successful 5-membered cyclisations *via* intramolecular epoxide openings (see Table 3.1) may be classified into two 'favoured' categories of cyclisation using Baldwin's analysis. Entries 1, 2 and 6 (Table 3.1) are 5-(*ENOLEXO*)-*EXO-TET* processes, whereas Entries 3, 4 and 5 are 5-*EXO-TET* cyclisations since the attacking orbitals of the nucleophilic atom are  $sp^2$  hybridised. Only the final, unsuccessful example (Entry 7) is of the same 'disfavoured'



5-(*ENOLENDO*)-*EXO-TET*' type as the cyclisation that we require. It also probably failed due to competition with 'favoured' processes.

The fact that our required reaction type (Path A) is 'disfavoured' by Baldwin's 'rules' does not preclude the possibility of it occurring under any circumstances. It simply means that 'disfavoured' processes have a kinetic handicap with respect to any 'favoured' reaction pathways which are available. Indeed reactions which are 'disfavoured' processes have been shown to proceed (see also Section 4.2.1).<sup>112-114</sup> In the case of 5-(*ENOLENDO*)-*EXO-TET*' cyclisations, however, we have been unable to find any examples of successful reactions. In addition, it must be recognised that the biochemical cyclisation which we were attempting to emulate does **not** involve the cyclisation of a simple ester, but rather a  $\beta$ -ketoester **2.12b**, (see Scheme 2.7). Thus the feasibility of using a glycidyl  $\beta$ -ketoester **3.25a** as a model cyclisation substrate was investigated.

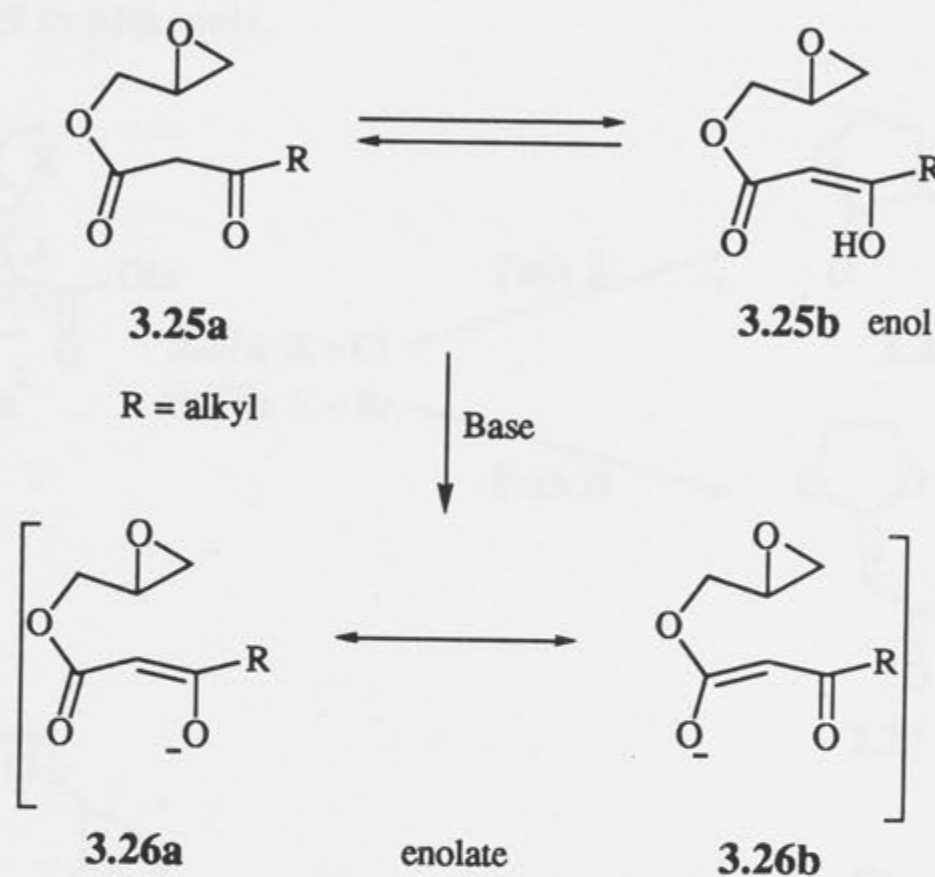


The cyclization of a glycidyl  $\beta$ -ketoester may be considered to be a hybrid of the two cyclizations shown in Scheme 3.11, in which the reaction is expected to proceed via *ENOLENDO* and *EXO-TET* pathways, respectively, to the ring as to be formed. The formation of 5-membered rings via intramolecular cyclization of an *ENOLENDO* system (cf. 3.18b) has been shown in the preceding discussion to be kinetically favoured. In contrast, *EXO-TET* cyclizations usually form 3-membered rings.<sup>115,116</sup> However, the relative rates of cyclization via *ENOLENDO* and *EXO-TET* pathways (and consequently the ability to cyclize) is not easy to predict. Conflicting reports regarding this aspect in the literature.

McDonald and Weaver<sup>117</sup> claim that the cyclization of the diester **3.27a** occurs through the *enolendo* pathway (Path A, Scheme 3.11) to give the bicyclic product.

### 3.1.4 Cyclisation of Glycidyl $\beta$ -Ketoesters

It was recognised that the fundamental difference between ester and  $\beta$ -ketoester cyclisation substrates is that, *a priori*, the latter have the ability to form *EXO* enols **3.25b** or *EXO* enolates **3.26a** as depicted in Scheme 3.11, thus potentially permitting cyclisation via a 'favoured' 5-(*ENOLEXO*)-*EXO*-*TET*' process.



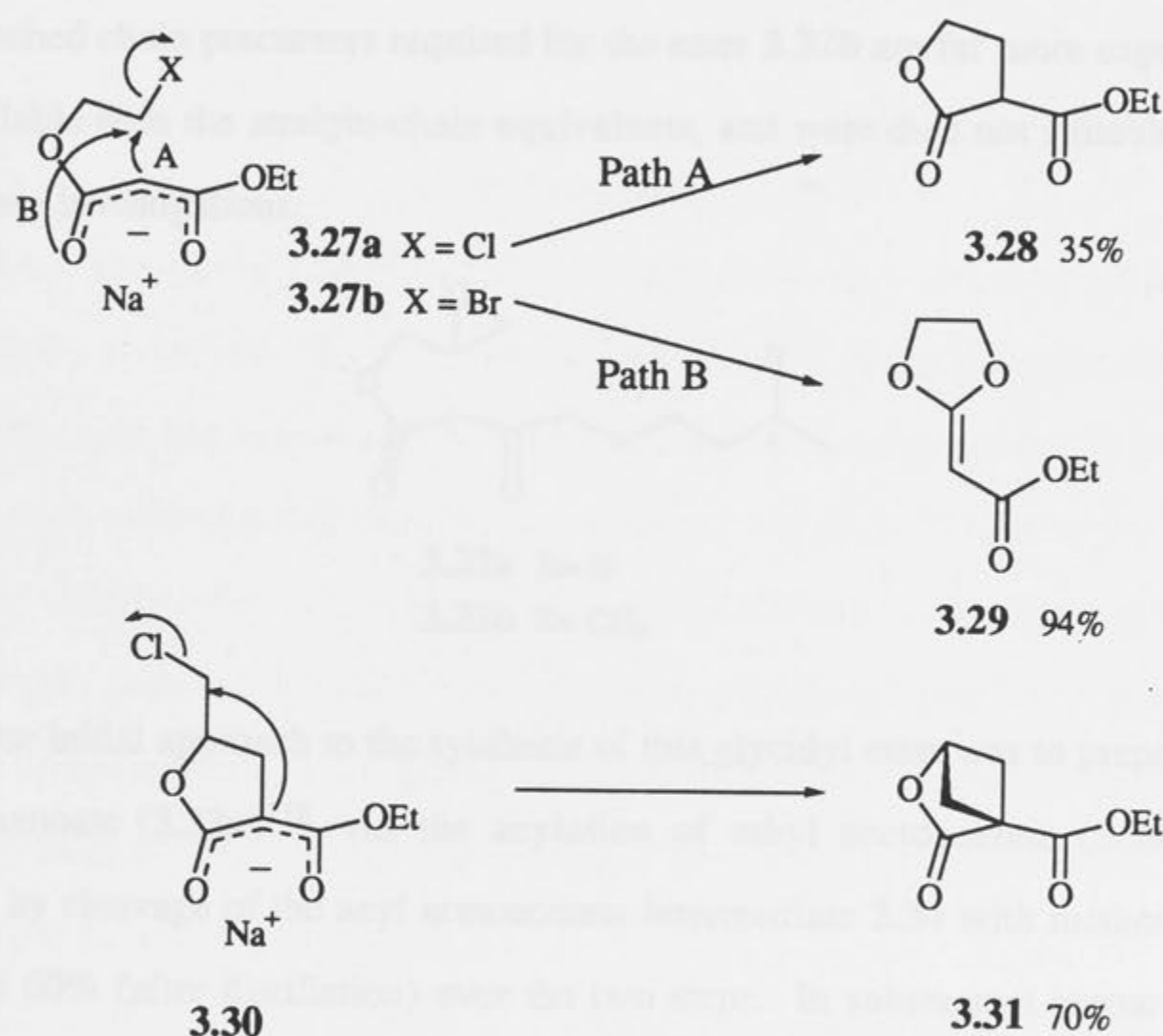
Scheme 3.11

The anion of a glycidyl- $\beta$ -ketoester may be considered to be a hybrid of the two canonical forms **3.26a** and **3.26b**, (Scheme 3.11) in which the enolate  $\pi$ -electron density is *EXO* and *ENDO*, respectively, to the ring to be formed. The formation of 5-membered rings via intramolecular alkylation of an *ENDO* enolate (*cf* **3.26b**) has been shown in the preceding discussion to be stereoelectronically 'disfavoured'. In contrast, *EXO* enolates readily form 5-membered rings.<sup>109,92</sup> However, the enolates of  $\beta$ -ketoesters incorporate both *EXO* and *ENDO*  $\pi$ -electron density and consequently their ability to cyclise is not easy to predict. Conflicting reports regarding this appear in the literature.

Michael and Weiner<sup>115</sup> claim that the sodium enolate of the chloroester **3.27a** cyclises through the enolate carbon (Path A, Scheme 3.12) to give the butyrolactone

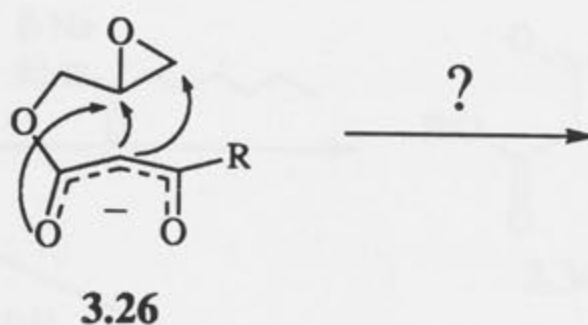


**3.28** albeit in low yield (35%). The identity of their product was said to have been confirmed by independent synthesis. In addition, they claim that the sodium enolate of the chloro  $\gamma$ -lactone **3.30** undergoes intramolecular C-alkylation to form the bicyclic lactone **3.31** in 70% yield. In contrast, Parker<sup>116</sup> reports (without reference to the earlier results of Michael and Weiner) that the sodium enolate of the corresponding bromoester **3.27b** cyclises exclusively *via* intramolecular O-alkylation to give the ketene acetal **3.29** in 94% yield.

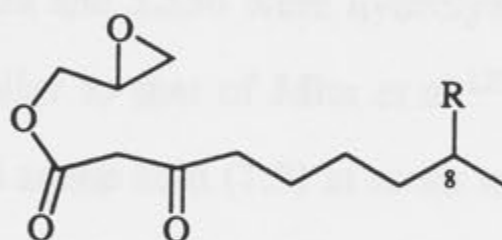


**Scheme 3.12 Literature Reports of  $\beta$ -Ketoester Cyclisations**

It seems unlikely that this dichotomy of results is due simply to the difference in leaving groups between **3.27a** and **3.27b** since, if anything, a bromide leaving group would be expected to enhance C-alkylation.<sup>117</sup> Thus, there exist conflicting literature precedents for the cyclisation behaviour of glycidyl- $\beta$ -ketoester substrates **3.26**.



To address this question of regioselectivity, the 3-oxononanoic ester **3.32a** was prepared. This straight chain substrate was chosen to provide a close model for the methyl-branched system **3.32b** which would be required for the preparation of A-factor. The branched chain precursors required for the ester **3.32b** are far more expensive and less available than the straight-chain equivalents, and were thus not suitable for these preliminary investigations.

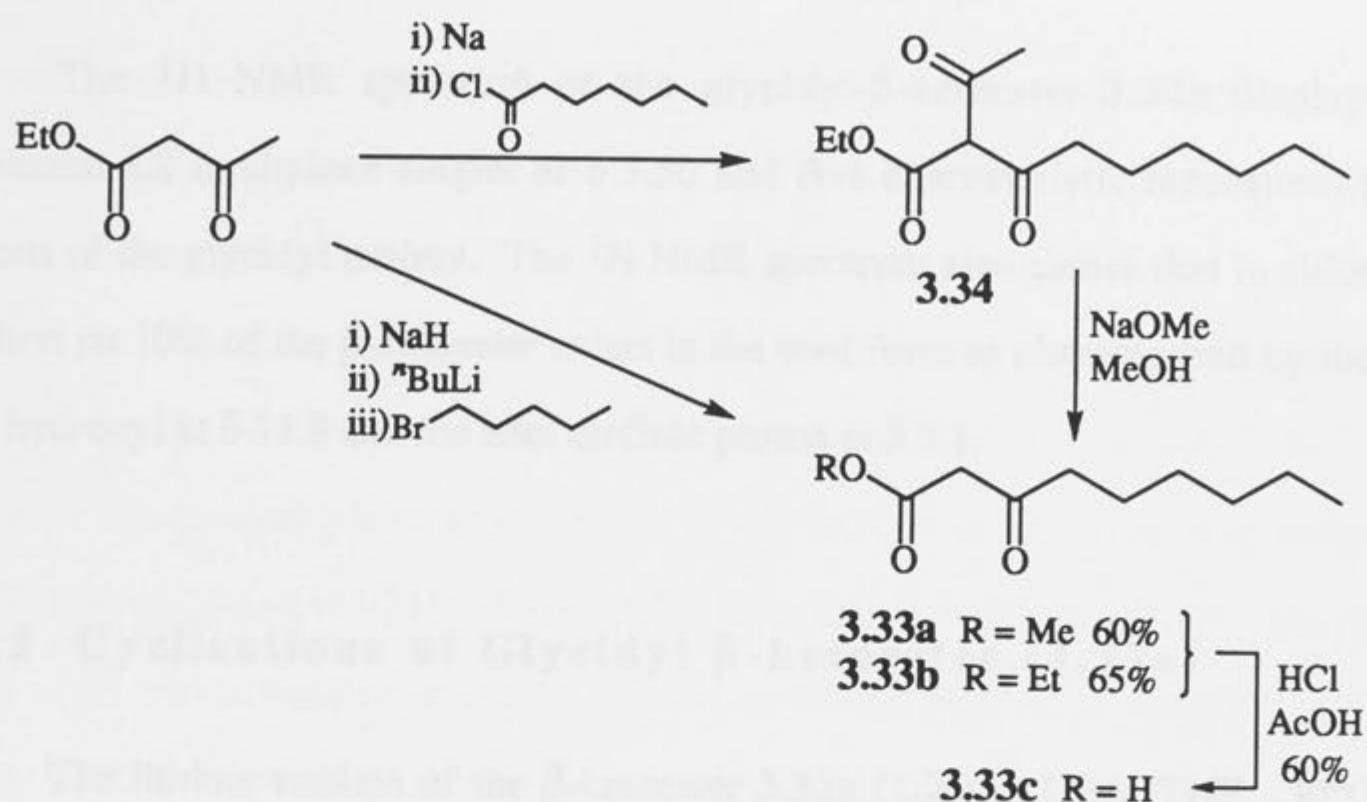


**3.32a** R= H

**3.32b** R= CH<sub>3</sub>

Our initial approach to the synthesis of this glycidyl ester was to prepare methyl 3-oxononanoate (**3.33a**)<sup>118</sup> *via* the acylation of ethyl acetoacetate (Scheme 3.13) followed by cleavage of the acyl acetoacetate intermediate **3.34** with methoxide. The yield was 60% (after distillation) over the two steps. In subsequent preparations we employed the more direct and faster method of Huckin and Weiler<sup>119</sup> (see Scheme 3.13) in which the dianion of ethyl acetoacetate was alkylated to give the ethyl  $\beta$ -ketoester **3.33b**, in similar yield (65%).

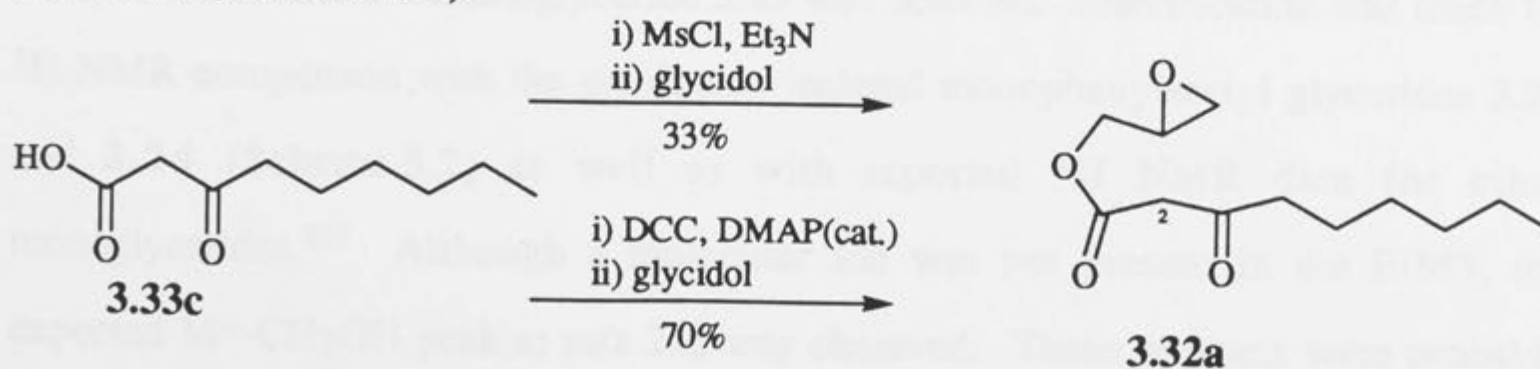




### Scheme 3.13

The  $\beta$ -ketoesters **3.33a** and **3.33b** were hydrolysed to the  $\beta$ -ketoacid **3.33c** in 60% yield by a method similar to that of Mitz *et al.*<sup>120</sup> using concentrated aqueous hydrochloric acid and glacial acetic acid (1:2) at room temperature for 22 hours. The crude product, which crystallised from the acid mixture, is *ca* 95% pure (by <sup>1</sup>H NMR) and can be esterified directly. However, if the product is to be stored, recrystallisation from benzene / hexane is recommended to prevent decarboxylation which appears to be promoted by impurities. This relatively old acid hydrolysis procedure is far superior to the base hydrolysis (K<sub>2</sub>CO<sub>3</sub> / MeOH) employed by modern workers<sup>121</sup> which gives low (20-30%) yields of unstable product.

Esterification of the  $\beta$ -ketoacid **3.33c** with glycidol was first attempted using mesyl chloride / triethylamine<sup>122</sup> but this gave only low yields (*ca* 33%) of the glycidyl  $\beta$ -ketoester **3.32a**. The use of N,N-dicyclohexylcarbodiimide (DCC) catalysed with 4-dimethylaminopyridine (DMAP)<sup>123</sup> improved the yield of ester to 70% after distillation (Scheme 3.14).



### Scheme 3.14



The  $^1\text{H}$  NMR spectrum of the glycidyl- $\beta$ -ketoester **3.32a** displayed the prominent C2 methylene singlet at  $\delta$  3.50 and five characteristic resonances for the protons of the glycidyl moiety. The  $^1\text{H}$  NMR spectrum also shows that in chloroform solution *ca* 10% of the  $\beta$ -ketoester exists in the enol form as characterised by the sharp enol hydroxyl at  $\delta$  11.8 and the enol olefinic proton at  $\delta$  5.1.

### 3.1.5 Cyclisations of Glycidyl $\beta$ -ketoester (**3.32a**)

The lithium enolate of the  $\beta$ -ketoester **3.32a** (1.2 eq., LDA, THF,  $-30^\circ\text{C}$ ) was found to be quite unreactive. Gas chromatographic analysis of the reaction mixture showed only unchanged starting material even after 20 hours at room temperature. Complete enolate formation was confirmed by GC and  $^1\text{H}$  NMR analysis of a sample of reaction mixture quenched with an excess of methyl iodide. Less than 5% of the sample was unmethylated starting material.

To eliminate the possibility discussed above that an H-bonded complex<sup>105</sup> between the enolate and the diisopropylamine could be inhibiting anion reactivity, we repeated the reaction with the non-amide base, sodium hydride. This base also allows the use of elevated reaction temperatures.

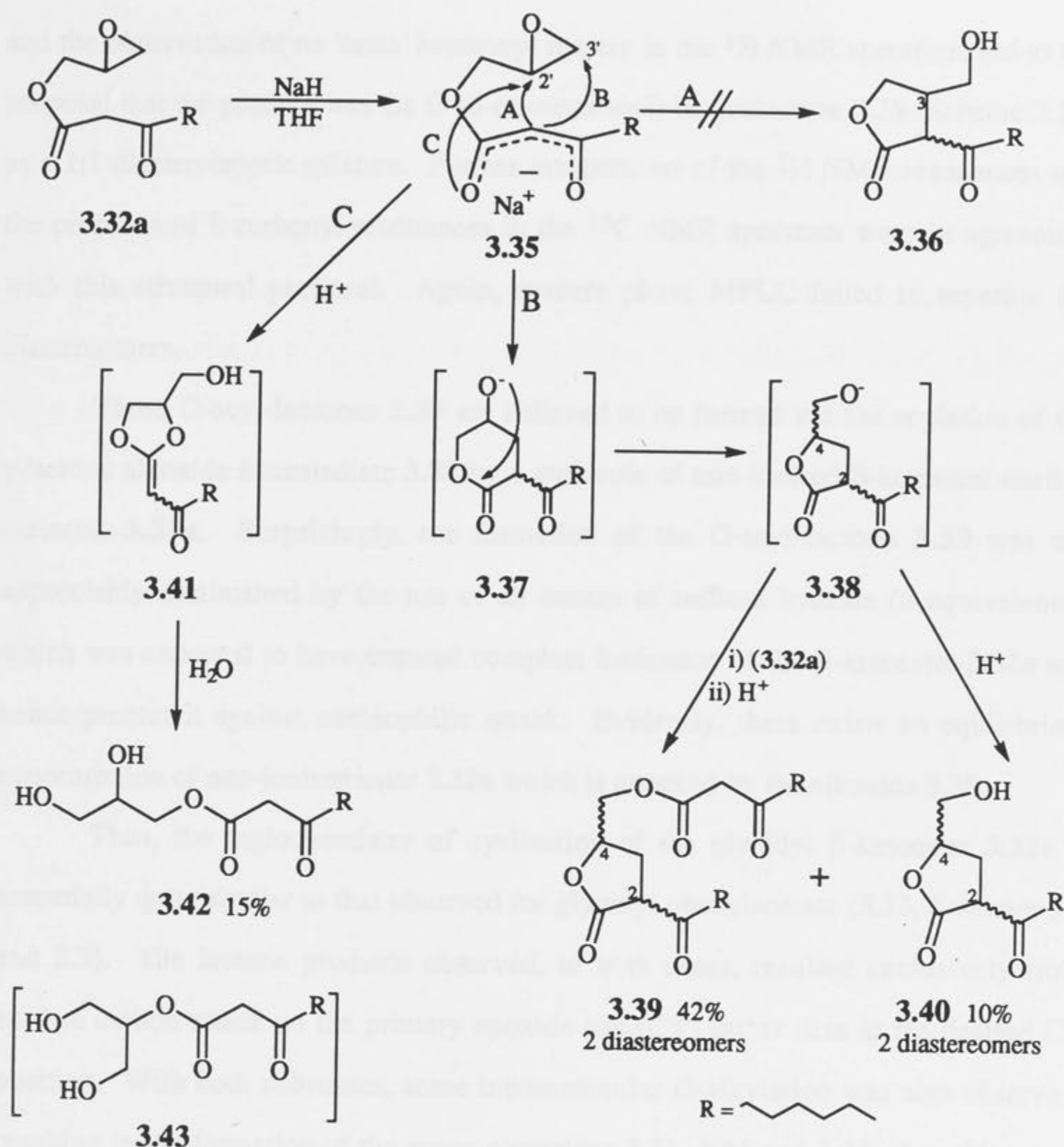
The sodium enolate of the  $\beta$ -ketoester **3.32a** (1.2 eq., NaH, THF,  $20^\circ\text{C}$ ) was also unreactive at room temperature. After heating at reflux for 3 hours three compounds were isolated from the reaction mixture using radial chromatography, with the remainder being complex polar material. The most polar product isolated, formed in 15% yield, was identified as the 1-monoglyceride **3.42** (Scheme 3.15). In this case none of the isomeric 2-monoglyceride **3.43** was detected. Identification was made by  $^1\text{H}$  NMR comparison with the previously isolated monophenylacetyl glycerides **3.23** and **3.24** (Scheme 3.7) as well as with reported  $^1\text{H}$  NMR data for other monoglycerides.<sup>107</sup> Although a molecular ion was not present in the EIMS, the expected  $\text{M}^+-\text{CH}_2\text{OH}$  peak at  $m/z$  215 was observed. These products were probably formed in a process directly analogous to that described for the monophenylacetyl



glycerides **3.23** and **3.24** (see Scheme 3.7) in which the enolate lactone oxygen intramolecularly opens the epoxide at C2' (Path C, Scheme 3.15), forming the intermediate ketene acetal **3.41**, which is subsequently hydrolysed.

The next most polar compound, formed in 10% yield, was identified as a  $\gamma$ -lactone by its characteristic infrared carbonyl stretching band at  $1780\text{ cm}^{-1}$ , in the centre of the normal  $\gamma$ -lactone range<sup>103</sup> of  $1795\text{--}1760\text{ cm}^{-1}$ . The observation of a second carbonyl band at  $1725\text{ cm}^{-1}$  suggested that a ketone group was still present. The EIMS displayed a weak peak at  $m/z$  228 which was confirmed as being the molecular ion by CIMS. High resolution mass measurement of this peak indicated a molecular composition of  $\text{C}_{12}\text{H}_{20}\text{O}_4$  which is consistent with either of the two hydroxymethyl-substituted  $\gamma$ -lactones **3.40** or **3.36**, both of which could be formed by intramolecular epoxide opening (Scheme 3.15). Although the  $^1\text{H}$  NMR spectrum (300 MHz) was very complex due to extensive coupling and the presence of a diastereomeric mixture, it allowed us to identify the product by comparison with the  $^1\text{H}$  NMR spectrum of the previously identified diastereomeric 4-hydroxymethyl butyrolactones **3.16** (Scheme 3.6). Again we observed the distinctive C4 methine multiplets between  $\delta$  4.58–4.73 which indicate a 4-hydroxymethyl substituent, rather than the C4 methylene protons of a 3-hydroxymethyl compound, which would be expected as doublets of doublets at *ca*  $\delta$  4.4 and  $\delta$  4.1.<sup>25</sup> The rest of the  $^1\text{H}$  NMR spectral data as well as the  $^{13}\text{C}$  NMR spectrum were fully consistent with the assignment of this product as a diastereomeric mixture of 4-hydroxymethyl butyrolactones **3.40**. These were presumably formed by enolate carbon attack at the primary end (C3') of the epoxide (Path B) followed by translactonisation and protonation, as depicted in Scheme 3.15. Attempts to separate the diastereomers by reverse-phase MPLC were unsuccessful.





Scheme 3.15

The least polar and major product, isolated in 42% yield, was also shown to be a  $\gamma$ -lactone by its IR carbonyl stretching band at  $1782\text{ cm}^{-1}$ . The largest ion detected in the EIMS was at  $m/z$  228, suggesting that this lactone was isomeric with the other  $\gamma$ -lactone product **3.40**. The possibility that this new lactone was the desired 3-hydroxymethyl butyrolactone **3.36** was eliminated when the CIMS revealed a molecular weight of 382, 154 mass units higher than required.  $^1\text{H}$  NMR spectroscopy revealed the distinctive multiplets at  $\delta$  4.67-4.84 indicating a 4-substituted butyrolactone structure as observed previously (*cf.* **3.40** and **3.16**). The appearance of two methylene singlets at  $\delta$  3.5 was characteristic of  $\beta$ -ketoester  $\alpha$ -methylene protons,



and the observation of an 'extra' heptanoyl moiety in the  $^1\text{H}$  NMR spectrum, led to the proposal that the product was the O-(3-oxononanoyl) butyrolactone **3.39** (Scheme 3.15) as a 1:1 diastereomeric mixture. Further assignments of the  $^1\text{H}$  NMR resonances and the presence of 8 carbonyl resonances in the  $^{13}\text{C}$  NMR spectrum were in agreement with this structural proposal. Again, reverse phase MPLC failed to separate the diastereomers.

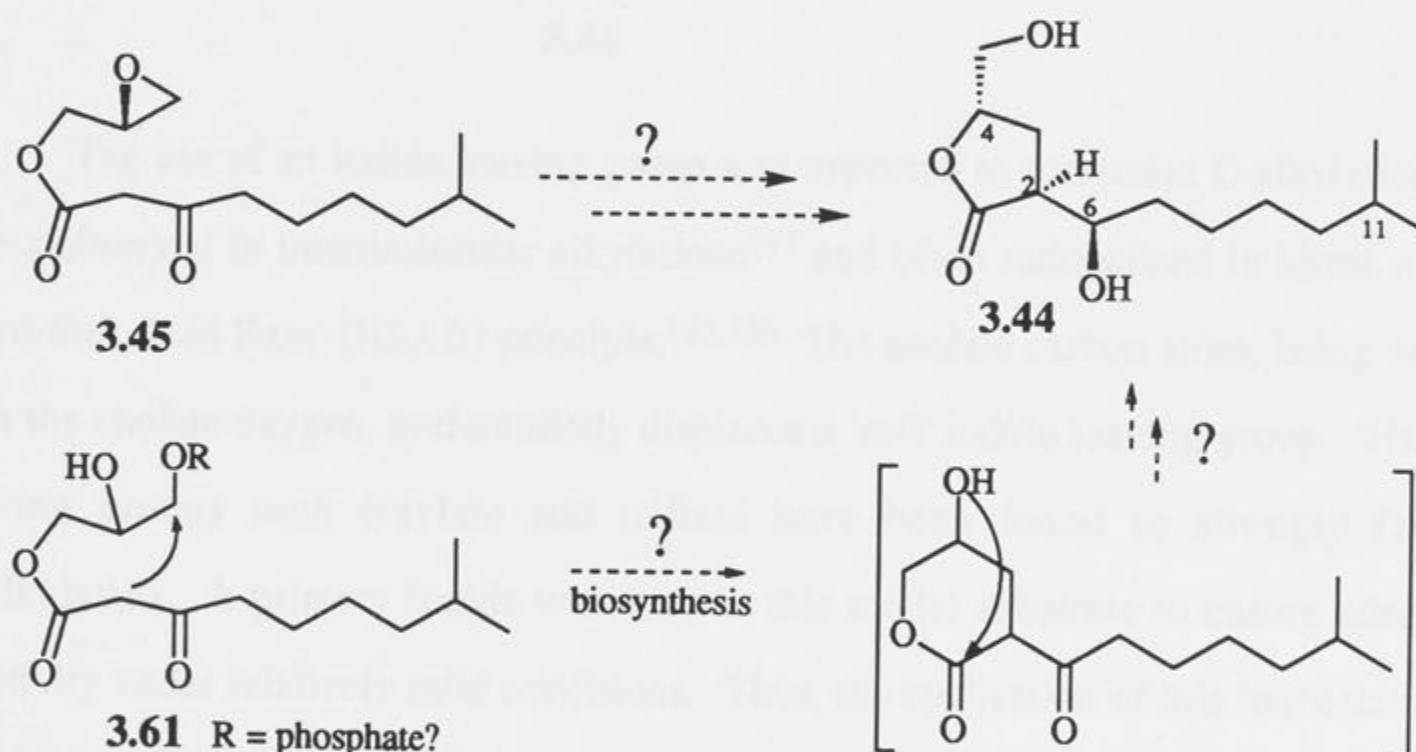
These O-acyl-lactones **3.39** are believed to be formed *via* the acylation of the  $\gamma$ -lactone alkoxide intermediate **3.38** by a molecule of non-ionised  $\beta$ -ketoester starting material **3.32a**. Surprisingly, the formation of the O-acyl-lactone **3.39** was not appreciably diminished by the use of an excess of sodium hydride (6 equivalents), which was expected to have ensured complete ionisation of the  $\beta$ -ketoester **3.32a** and hence protect it against nucleophilic attack. Evidently, there exists an equilibrium concentration of non-ionised ester **3.32a** which is attacked by the alkoxide **3.38**.

Thus, the regiochemistry of cyclisation of the glycidyl  $\beta$ -ketoester **3.32a** is essentially quite similar to that observed for glycidyl phenylacetate (**3.13**, Schemes 3.6 and 3.7). The lactone products observed, in both cases, resulted exclusively from enolate carbon attack on the primary epoxide site (C3') rather than at the desired C2' position. With both substrates, some intramolecular O-alkylation was also observed, resulting in the formation of the mono glycerides **3.23**, **3.24** and **3.42**. In neither case was there any evidence of the desired mode of cyclisation taking place. Base promoted cyclisations of glycidyl esters did not appear to be very promising. Acid catalysed cyclisations did not seem any more promising since the stereoelectronic effects involved in the attack of an ester enol (e.g. **3.25b**, see Scheme 3.11) on a protonated (or Lewis acid complexed) epoxide are identical to the reactions explored above.

Our interest in the cyclisation of glycidyl- $\beta$ -ketoesters has recently been rekindled following the isolation by Pathirana and co-workers<sup>124</sup> of a novel butanolide **3.44** from a marine Actinomycete which has been shown to have antibiotic properties. This metabolite is a 4-hydroxymethyl butyrolactone, very close in structure to the lactones **3.40** which we have isolated (see Scheme 3.15). It differs only by its oxidation



level at C6 and the presence of a C11 methyl substituent. The cyclisation of the glycidyl  $\beta$ -ketoester **3.45** potentially offers a very direct route to this antibiotic which may provide an alternative to the low-yielding route employed by its discoverers for their structural confirmation. The route could potentially model the biosynthesis of the metabolite **3.44** which may be achieved *via* the cyclisation and rearrangement of a  $\beta$ -ketoester of the common metabolite glycerol-1-phosphate (*i.e.* **3.61**, see Scheme 3.16). For a synthesis of this type to be viable, however, further work would be required to increase the yield of 4-hydroxymethyl butyrolactone **3.40** by the minimisation of the O-acylation of the alkoxide intermediate **3.38**. Stereocontrol at C4 could potentially be achieved with the use of optically active glycidol.

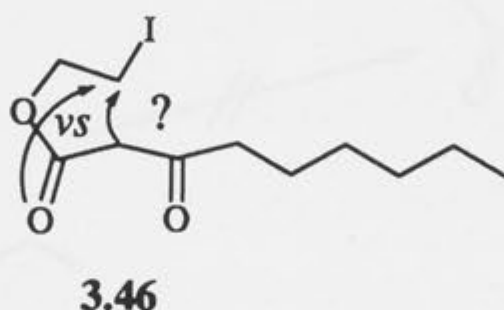


Scheme 3.16



### 3.1.6 Cyclisation of Iodo- $\beta$ -ketoester (3.46)

Frustrated by attempts to achieve intramolecular enolate carbon attack at the C2' position of glycidyl  $\beta$ -ketoester (*e.g.* Scheme 3.15, Path A), we prepared the iodoethyl  $\beta$ -ketoester **3.46** as a model cyclisation substrate which offers only a single reaction site. This substrate provides direct competition between intramolecular C- and O-alkylation by the  $\beta$ -ketoester enolate.

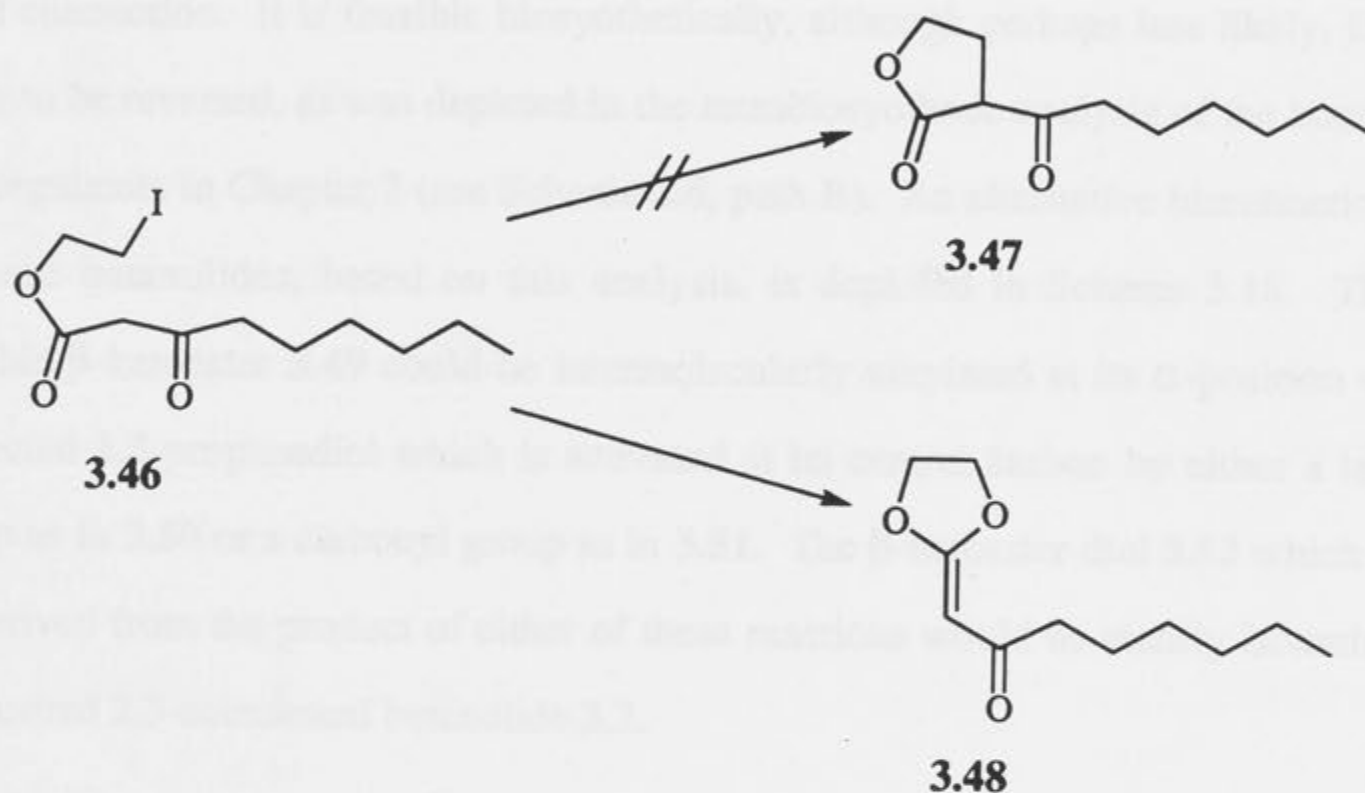


The use of an iodide leaving group was expected to maximise C-alkylation, an effect observed in intermolecular alkylations<sup>117</sup> and often rationalised in terms of the 'Hard-Soft Acid Base' (HSAB) principle.<sup>125,126</sup> The enolate carbon atom, being 'softer' than the enolate oxygen, preferentially displaces a 'soft' iodide leaving group. 'Harder' leaving groups such as tosylate and triflate have been found to strongly favour O-alkylation. A primary iodide was used in this model substrate to ensure adequate reactivity under relatively mild conditions. Thus, the cyclisation of this iodoester **3.46** should provide a clear test of the viability of forming  $\gamma$ -lactones *via* an intramolecular C-alkylation.

Esterification of 3-oxononanoic acid **3.33c** with iodoethanol formed the iodide (**3.46**). Treatment of this product with sodium hydride (THF, 20°C, 5 min) resulted in rapid and exclusive oxygen alkylation, forming the ketene acetal **3.48** in 75% yield (Scheme 3.17). The acetal was, in this case, sufficiently stable to isolate using silica chromatography. No sign of  $\gamma$ -lactone products was observed by <sup>1</sup>H NMR spectroscopy in any of the chromatographic fractions. This result conflicts with the report of Michael and Weiner<sup>24</sup> who claim  $\gamma$ -lactone products **3.28** and **3.31**, from related substrates **3.27** and **3.30** respectively, (Scheme 3.12). However, it is consistent with the report of Parker<sup>116</sup> and that of Svendsen *et al.*<sup>127</sup> who also observed exclusive

intramolecular O-alkylation in closely related 5-membered cyclisations (*e.g.* 3.27b, Scheme 3.12).

The reaction was repeated using tetra-*n*-butylammonium fluoride as base, a reagent specially recommended for the promotion of C-alkylation in intramolecular aldol condensations of  $\beta$ -ketoesters.<sup>128</sup> Again, we observed rapid and exclusive enolate oxygen attack to produce the ketene acetal 3.48 in 70% yield.



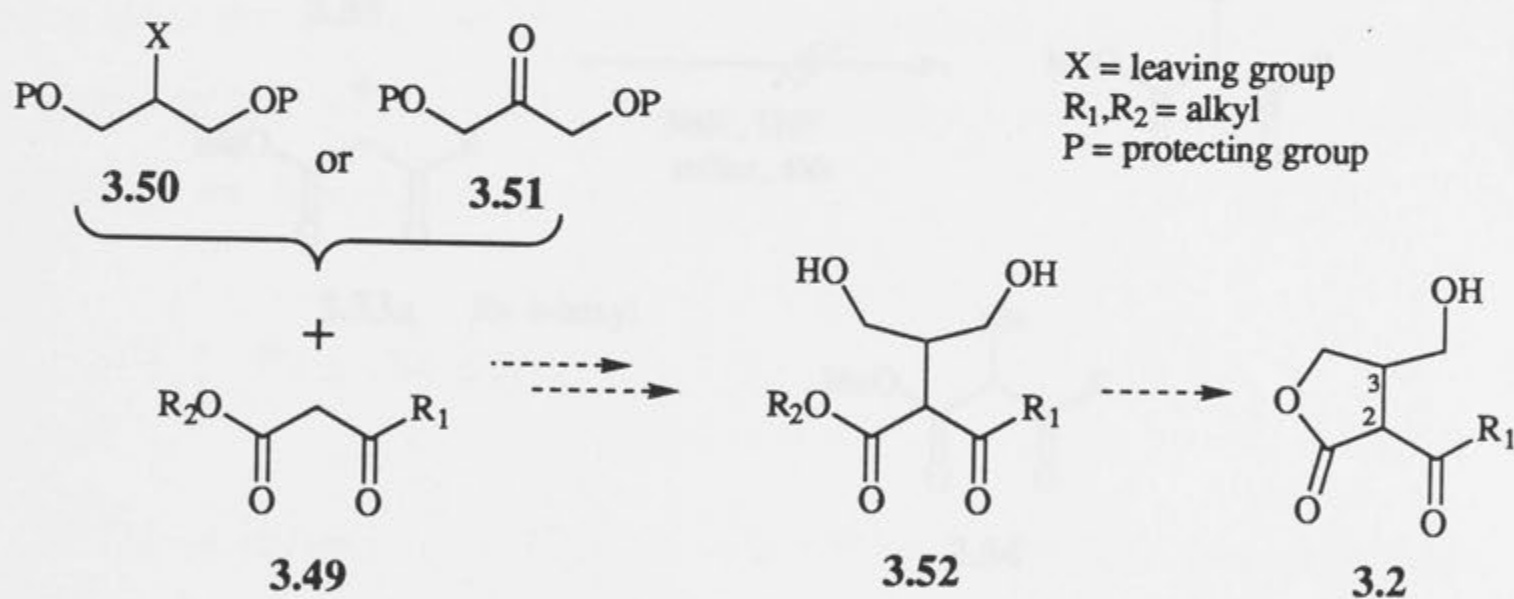
Scheme 3.17

The fact that we had failed to observe any of the desired intramolecular C-alkylation of  $\beta$ -ketoesters, even with this last promising substrate, led to the conclusion that the facile 'favoured' O-alkylation reaction pathway would dominate the reactivity of these systems, regardless of the leaving group employed. Apparently the  $\beta$ -ketoester enolates retain sufficient electron density in their *ENDO* C=C bond to ensure planarity across the enolate system and thus preclude reaction *via* a 'favoured' *ENOLEXO* process. At this stage we decided to discontinue further attempts to form  $\gamma$ -lactones by this method and instead investigate *intermolecular* alkylations as a method of forming  $\gamma$ -lactones which does not have such severe stereoelectronic constraints.



### 3.2 INTERMOLECULAR ALKYLATIONS OF $\beta$ -KETOESTERS

The experimental work thus far reflects attempts to model the biosynthetic construction of 2,3-disubstituted butanolides in which esterification precedes C2-C3 bond connection. It is feasible biosynthetically, although perhaps less likely, for this order to be reversed, as was depicted in the retrobiosynthetic analysis of the butanolide autoregulators in Chapter 2 (see Scheme 2.6, path B). An alternative biomimetic route to these butanolides, based on this analysis, is depicted in Scheme 3.18. Thus, a suitable  $\beta$ -ketoester **3.49** could be intermolecularly alkylated at its  $\alpha$ -position with a protected 1,3-propanediol which is activated at its central carbon by either a leaving group as in **3.50** or a carbonyl group as in **3.51**. The  $\beta$ -ketoester diol **3.52** which could be derived from the product of either of these reactions would be readily lactonised to the desired 2,3-substituted butanolide **3.2**.

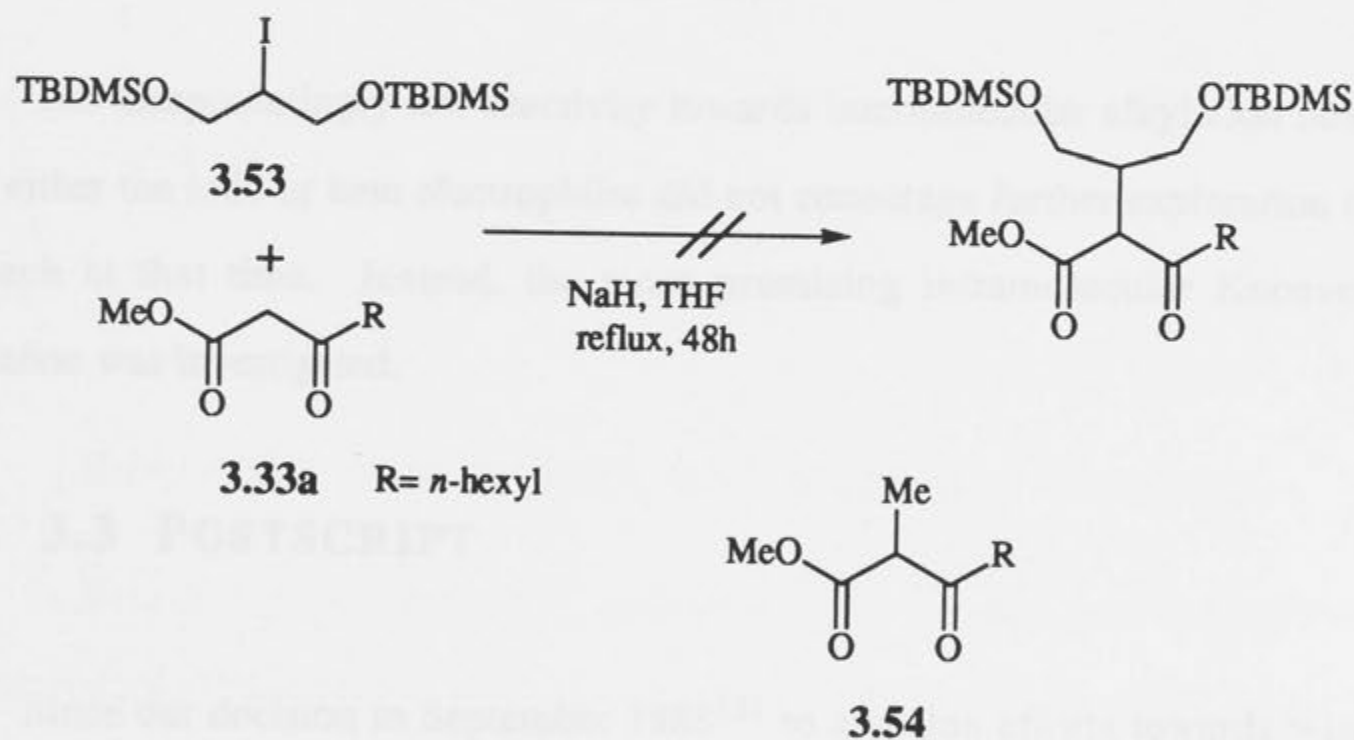


Scheme 3.18

While the initial alkylation step in a synthesis based on this analysis would lack the large entropic advantage of intramolecular reactions, the method would avoid the associated severe stereoelectronic constraints which we have encountered, since the ring closure step, a lactonisation, would now be of the 'favoured' 5-*EXO-TRIG* type.

To assess the feasibility of this approach, the diprotected iodo-diol **3.53** was prepared by treatment of 2-iodopropanediol<sup>129,130</sup> with TBDMS-triflate. Iodide was again chosen as the leaving group due to its high reactivity and its reported ability to maximise C-alkylation of  $\beta$ -ketoester enolates.<sup>117</sup>

When the iodide was heated at reflux in THF for 48 hours with the sodium enolate of the  $\beta$ -ketoester (**3.33a**), no sign of reaction was detected by TLC analysis. That the anion was still active after this time was demonstrated by the addition of an excess of methyl iodide to the reaction mixture. The  $\alpha$ -methyl- $\beta$ -ketoester **3.54** was isolated in 80% yield, (Scheme 3.19). That the enolate was C- rather than O-methylated was determined by the observation in the  $^1\text{H}$  NMR spectrum of a methine quartet at  $\delta$  3.52 and a doublet methyl resonance at  $\delta$  1.33, features expected only for the C-alkylation product **3.54**. In addition, the unreacted iodopropanediol **3.53** was recovered almost quantitatively (96%).

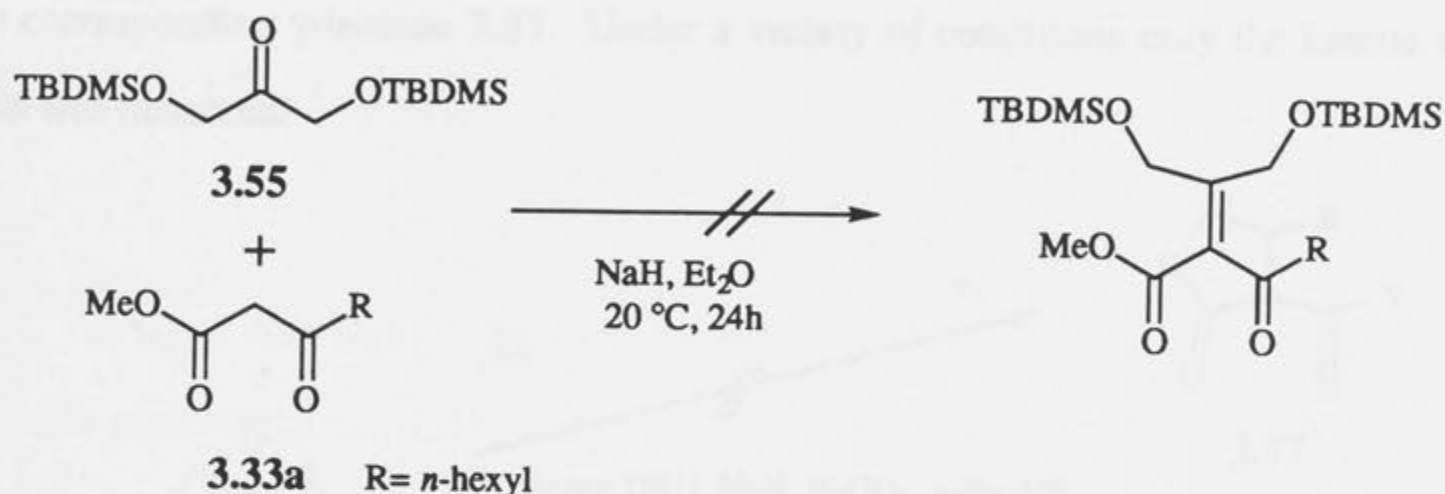


Scheme 3.19

The lack of reactivity displayed by this sterically crowded secondary iodide, even under these rather forcing conditions, prompted us to examine the analogous reaction using the ketonic electrophile **3.55**. Dihydroxyacetone was diprotected as **3.55** and was added to a solution of the sodium enolate of the  $\beta$ -ketoester (**3.33a**) in diethyl ether. After 24 hours at room temperature the silyl ketone had been wholly converted into a mixture of complex products whereas the ester **3.33a** was largely recovered



(90%) by chromatography (Scheme 3.20). The mixture of products, although not separated or fully identified, was shown by  $^1\text{H}$  NMR to lack the characteristic resonances of hexyl or ethoxyl moieties which would be expected to be present if any of the components had been derived *via* Knoevenagel condensation with the  $\beta$ -ketoester 3.33a. The by-products presumably result from base catalysed self-condensation of the silyl ketone.



Scheme 3.20

The disappointingly low reactivity towards intermolecular alkylation observed using either the iodo or keto electrophiles did not encourage further exploration of this approach at that time. Instead, the more promising intramolecular Knoevenagel cyclisation was investigated.

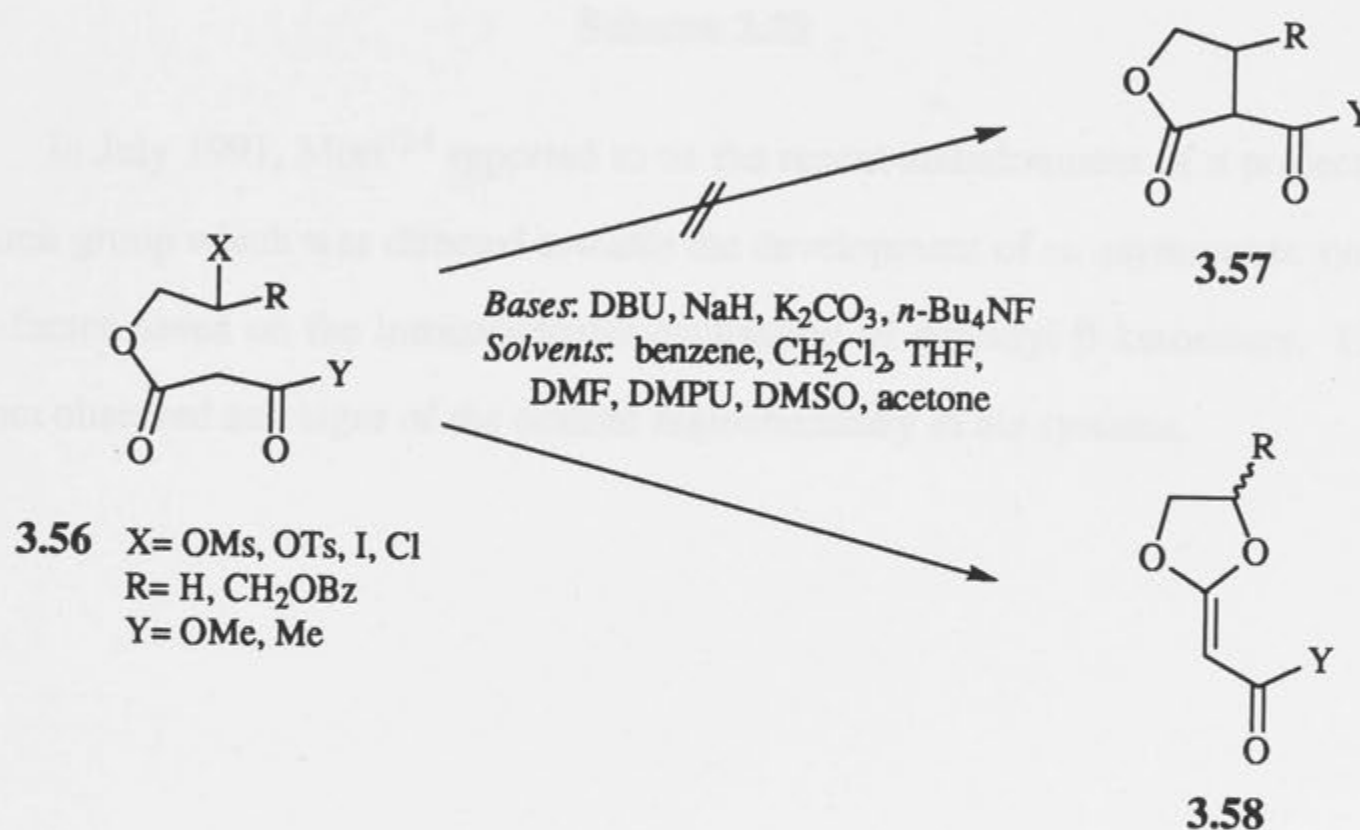
### 3.3 POSTSCRIPT

Since our decision in September 1988<sup>131</sup> to abandon efforts towards  $\gamma$ -lactone formation *via* intramolecular enolate alkylation, several reports of closely related investigations have been received which fully vindicate the change of strategy.

In mid-1990, Adams *et al.*<sup>132</sup> published a report of their many attempts to form  $\gamma$ -lactones 3.57 *via* the intramolecular alkylation of a variety of  $\beta$ -ketoesters and malonic esters (3.56, Scheme 3.21), with the intention of synthesising lactone-containing natural products including A-factor (1.1). Despite the range of leaving groups, bases and solvents utilised they failed to detect any sign of  $\gamma$ -lactone products. They concluded:

"Regardless of a) the base, b) the solvent, c) the leaving group, and d) the substitution degree at the electrophilic reaction site ( $1^\circ$  vs  $2^\circ$ ), only O-alkylation at the proximal CO group has been observed giving ketene acetals" (3.58).

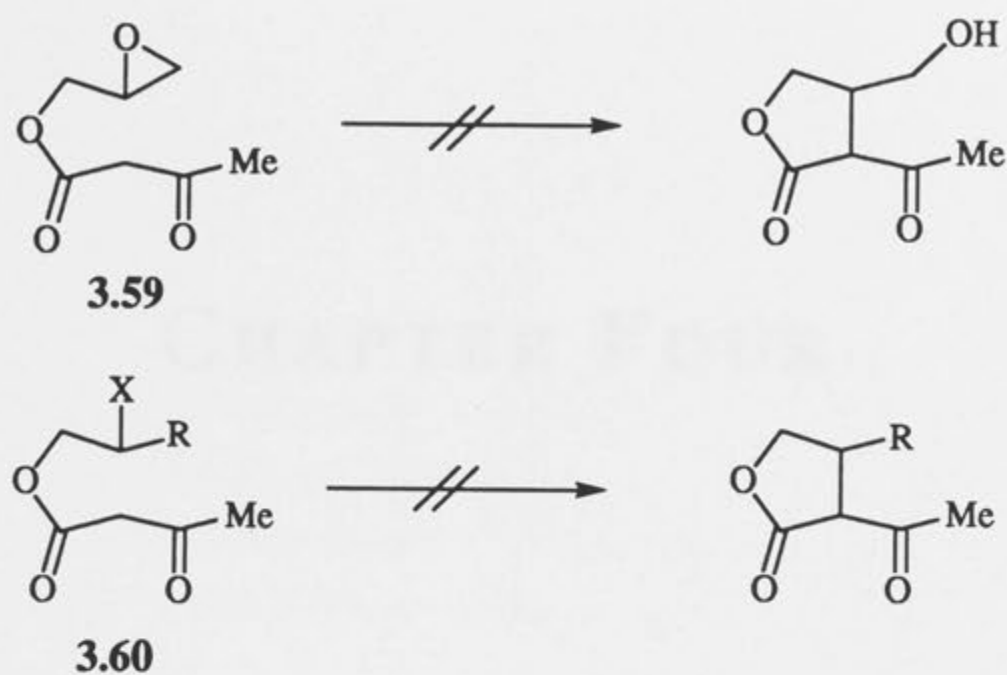
Their report also describes their unsuccessful attempts to repeat the work of Michael and Weiner<sup>115</sup> who allegedly cyclised the chloroester 3.56 ( $X = \text{Cl}$ ,  $R = \text{H}$ ,  $Y = \text{OEt}$ ) to form the corresponding  $\gamma$ -lactone 3.57. Under a variety of conditions only the ketene acetal 3.58 was observed.



Scheme 3.21 Exclusive O-Alkylation Reported by Adams *et al.*

In February 1990 Yamada<sup>133</sup> informed us of his unsuccessful attempts to produce A-factor analogues by methods almost identical to ours, in which he used both epoxide 3.59 and acyclic 3.60 electrophiles (Scheme 3.22).





Scheme 3.22

In July 1991, Mori<sup>134</sup> reported to us the recent abandonment of a project in his research group which was directed towards the development of an asymmetric synthesis of A-factor based on the intramolecular cyclisation of glycidyl- $\beta$ -ketoesters. He also had not observed any signs of the desired regiochemistry in his systems.

## CHAPTER FOUR

### THE BIOMIMETIC SYNTHESIS OF A-FACTOR

*If one way is better than another,  
that you may be sure is Nature's way.*

Aristotle (384-322 BC)

Nicomachean Ethics 1099B, 23

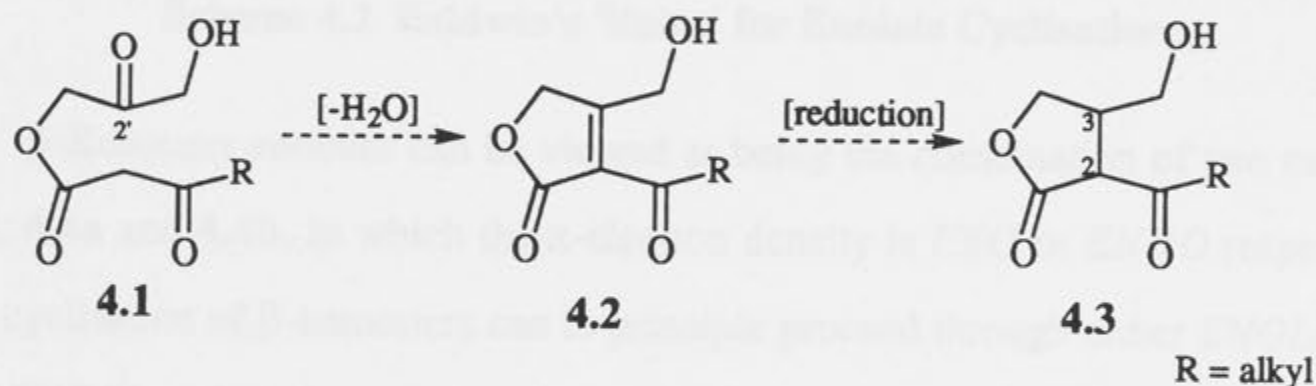


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## 4.1 $\gamma$ -LACTONES *via* INTRAMOLECULAR KNOEVENAGEL CONDENSATION?

Incorporation of a carbonyl group rather than a leaving group at C2' of a  $\beta$ -ketoester, as in **4.1**, could allow cyclisation *via* Knoevenagel condensation (alternative intramolecular route Aii, Scheme 2.9). This process also models one of the proposed biosynthetic pathways to the butanolide autoregulators (see Chapter 2, Scheme 2.8). Base-promoted intramolecular Knoevenagel condensation would give the  $\alpha,\beta$ -unsaturated lactone **4.2**, which could then be reduced to form butanolides **4.3** as depicted in Scheme 4.1. Our initial investigation of this approach explored the feasibility of the key cyclisation step.



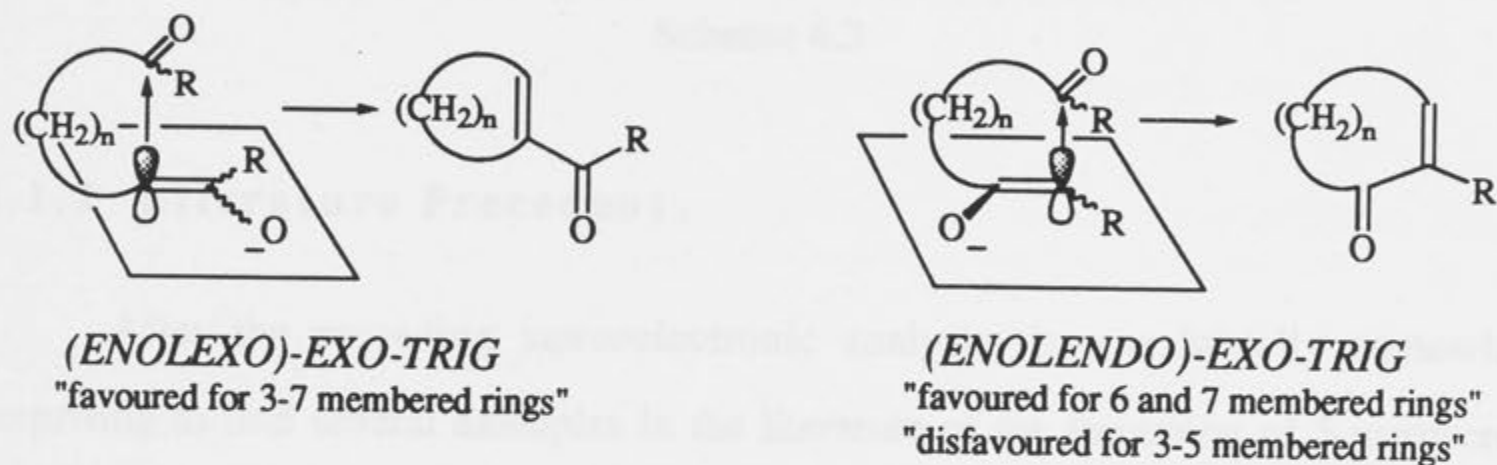
Scheme 4.1

### 4.1.1 Stereoelectronic Considerations

The stereoelectronic requirements for intramolecular attack of a  $\beta$ -ketoester enolate carbon at a trigonal electrophilic centre are related to those previously discussed in Section 3.1.2 for a tetrahedral electrophile. However, the  $\pi$  orbitals of the enolate must approach a trigonal centre at a different angle to that in the attack of a tetrahedral centre (*ca*  $109^\circ$  to the C=O axis rather than *ca*  $180^\circ$  to the C-LG bond).<sup>135</sup> Nevertheless, examination of Dreiding molecular models shows that this difference does not appear to affect greatly the strain involved in achieving the geometry required for reaction. In both cases, the amount of strain appears to depend mostly on the length and rotational flexibility of the chain of atoms linking the reacting sites. Accordingly, it

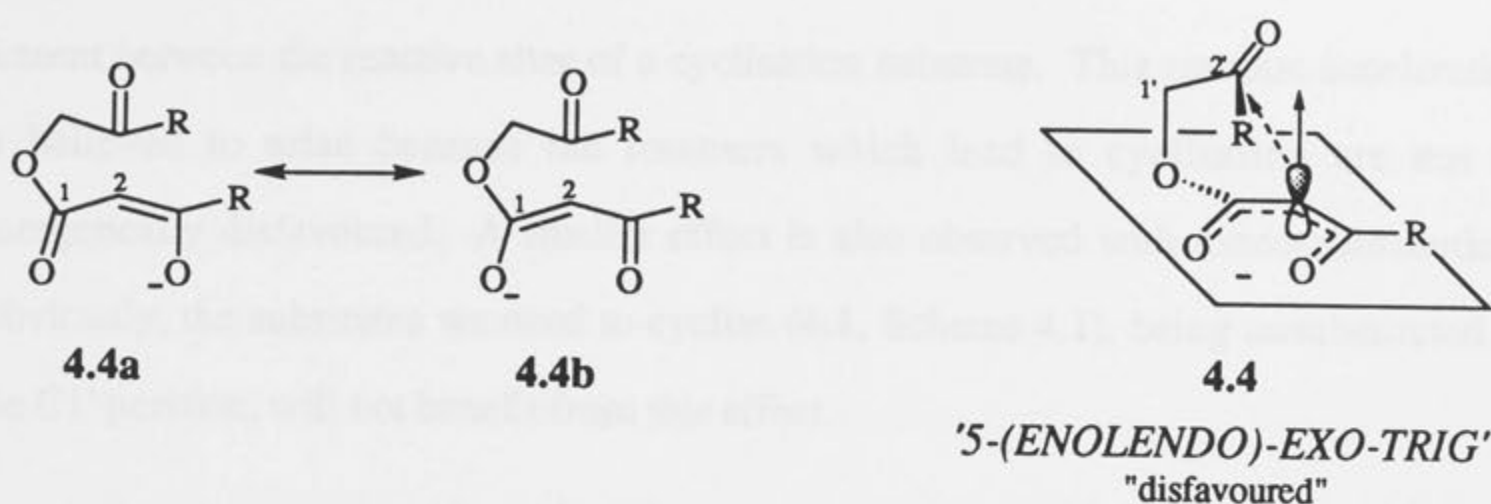


is understandable that Baldwin's 'rules'<sup>109</sup> for enolate cyclisations at a trigonal centre, summarised in Scheme 4.2, are the same as those previously discussed for tetrahedral cases (Scheme 3.8). Thus cyclisations to form 5-membered rings will only be stereoelectronically 'favoured' if the enolate  $\pi$ -electron density in the substrate is *EXO* to the ring being formed and therefore does not restrict the rotational freedom of the ring-forming chain of atoms (*i.e.* a 5-(*ENOLEXO*)-*EXO-TRIG* process).



**Scheme 4.2 Baldwin's 'Rules' for Enolate Cyclisations**

$\beta$ -Ketoester enolates can be viewed as being the combination of two canonical forms, **4.4a** and **4.4b**, in which the  $\pi$ -electron density is *EXO* or *ENDO* respectively. Thus, cyclisation of  $\beta$ -ketoesters can in principle proceed through either *ENOLEXO* or *ENOLENDO* modes. It was recognised, however, that any contribution of the latter canonical form **4.4b** will promote planarity of the enolate system, so as to hinder the freedom of rotation about the C1-C2 bond which is necessary for the 'favoured' *ENOLEXO* cyclisation process. The mesomeric delocalised anion **4.4** is therefore likely to stereoelectronically resemble a substrate for the 'disfavoured' 5-(*ENOLENDO*)-*EXO-TRIG* process, as depicted in Scheme 4.3. Hence,  $\gamma$ -lactone formation *via* intramolecular aldol-style condensation of  $\beta$ -ketoesters appears, stereoelectronically at least, to be no more favoured than the previously explored intramolecular alkylation approach.

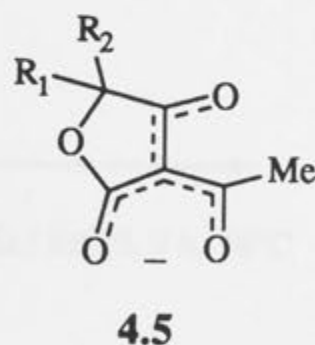


Scheme 4.3

#### 4.1.2 Literature Precedent

After the preceding stereoelectronic analysis, it was initially somewhat surprising to find several examples in the literature of the formation of 5-membered cyclic carbonyl compounds *via* the intramolecular attack of enolates on carbonyl groups (Table 4.1).

The syntheses of acyl tetronic acids (Table 4.1, Entries 1a, 1b and 1c) illustrate that when an ester carbonyl is the electrophile (*i.e.* Dieckmann condensation),  $\gamma$ -lactones may be formed. However, the elimination of alkoxide following cyclisation and the subsequent formation of the highly stabilised anion **4.5** under the basic conditions may give assistance to this type of cyclisation which is not available in our system.

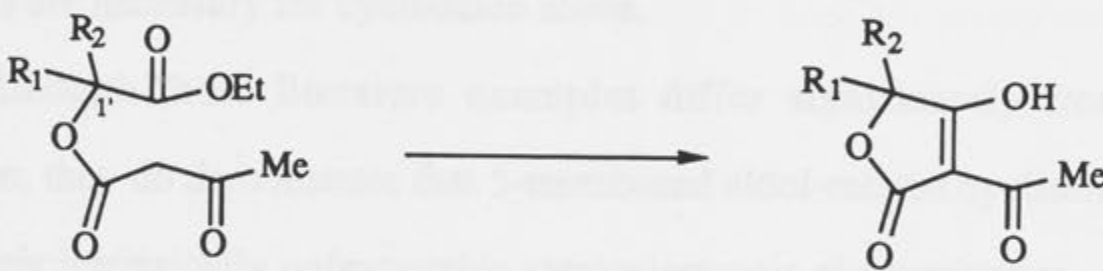
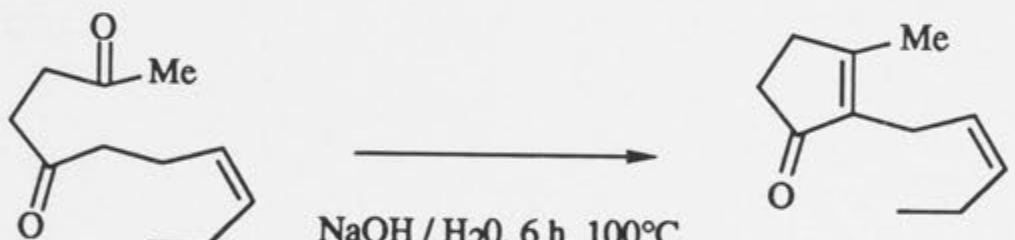
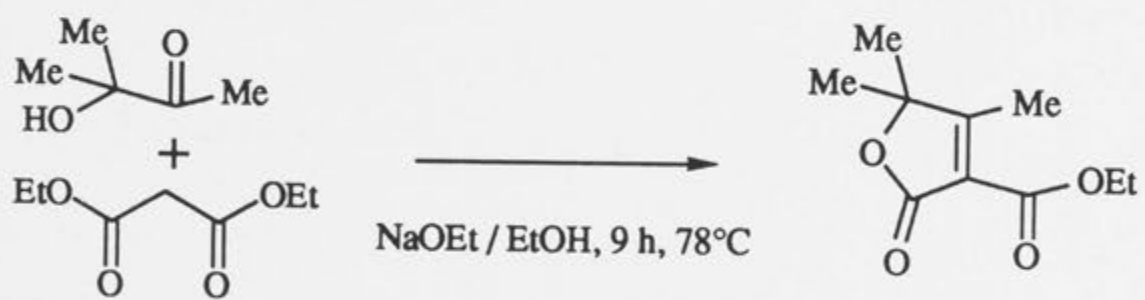


In addition, these reactions require much more forceful conditions when the C1' position is unsubstituted (Table 4.1, Entry 1c), relative to mono- or dimethylated examples (Table 4.1, Entry 1a and 1b). The methyl substituents presumably assist cyclisation by the so called '*gem*-dialkyl' effect,<sup>136</sup> a term used to describe the observed rate enhancements of up to 1000 fold which result when *gem*-dialkyl substituents are



present between the reactive sites of a cyclisation substrate. This reaction acceleration is believed to arise because the rotamers which lead to cyclisation are not so energetically disfavoured. A smaller effect is also observed with mono-substitution. Obviously, the substrates we need to cyclise (4.1, Scheme 4.1), being unsubstituted in the C1' position, will not benefit from this effect.

**Table 4.1 Examples of 5-Membered Ring Formation *via* Intramolecular Aldol-style Condensations**

Entry	Reaction	Yield	Ref.
1			
1a	$R_1, R_2 = \text{Me}$ Na / PhMe, 6 h, 20°C	67%	137
1b	$R_1 = \text{H}, R_2 = \text{Me}$ Na / PhMe, 6 h, 20°C	50%	137
1c	$R_1, R_2 = \text{H}$ NaOt-Bu / <i>t</i> -BuOH, 24 h, 82°C	80%	137
2		75%	138
3		63%	139

In the second entry of Table 4.1, an enolate attacks a ketone carbonyl, as we require, but the conditions necessary for reaction ( $\text{NaOH}_{\text{aq}}$ , 6 h,  $100^\circ\text{C}$ ) are probably too vigorous for more sensitive substrates such as esters. Since this substrate cannot form an *EXO* enol, it demonstrates that the stereoelectronically 'disfavoured' 5-(*ENOLENDO*)-*EXO-TRIG* process can occur, albeit under forcing conditions.

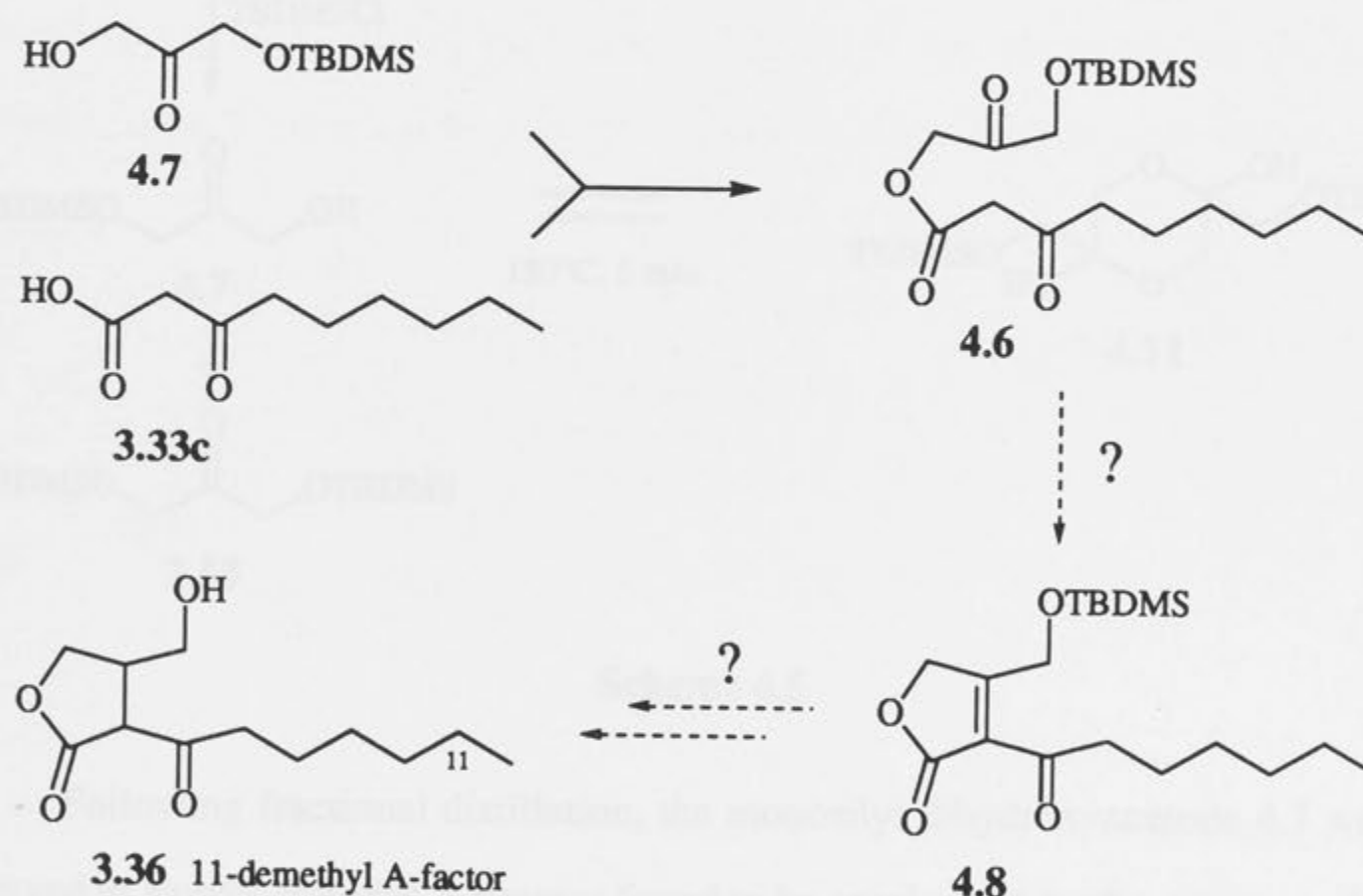
In the third entry of Table 4.1, the transesterification of diethyl malonate precedes an intramolecular Knoevenagel condensation similar to that required for the proposed synthesis. However, this cyclisation too is assisted by the '*gem*-dialkyl' effect and, since two reactions are combined in one rather vigorous step, it is not known what conditions are necessary for cyclisation alone.

Although these literature examples differ significantly from our required cyclisation, they do demonstrate that 5-membered aldol-related cyclisations can proceed despite their intrinsically unfavourable stereoelectronic characteristics.



## 4.2 SYNTHESIS OF DEMETHYL A-FACTOR VIA INTRAMOLECULAR KNOEVENAGEL CONDENSATION

The silyl protected straight-chain  $\beta$ -ketoester **4.6** was chosen as a readily accessible substrate on which to test the intramolecular Knoevenagel cyclisation. If successful, the butenolide product **4.8** could be elaborated to 11-demethyl A-factor **3.36** by reduction of the tetra-substituted olefinic moiety and desilylation. It was thought prudent to incorporate TBDMS-protection in the cyclisation precursor **4.6** to minimise undesired reactivity.



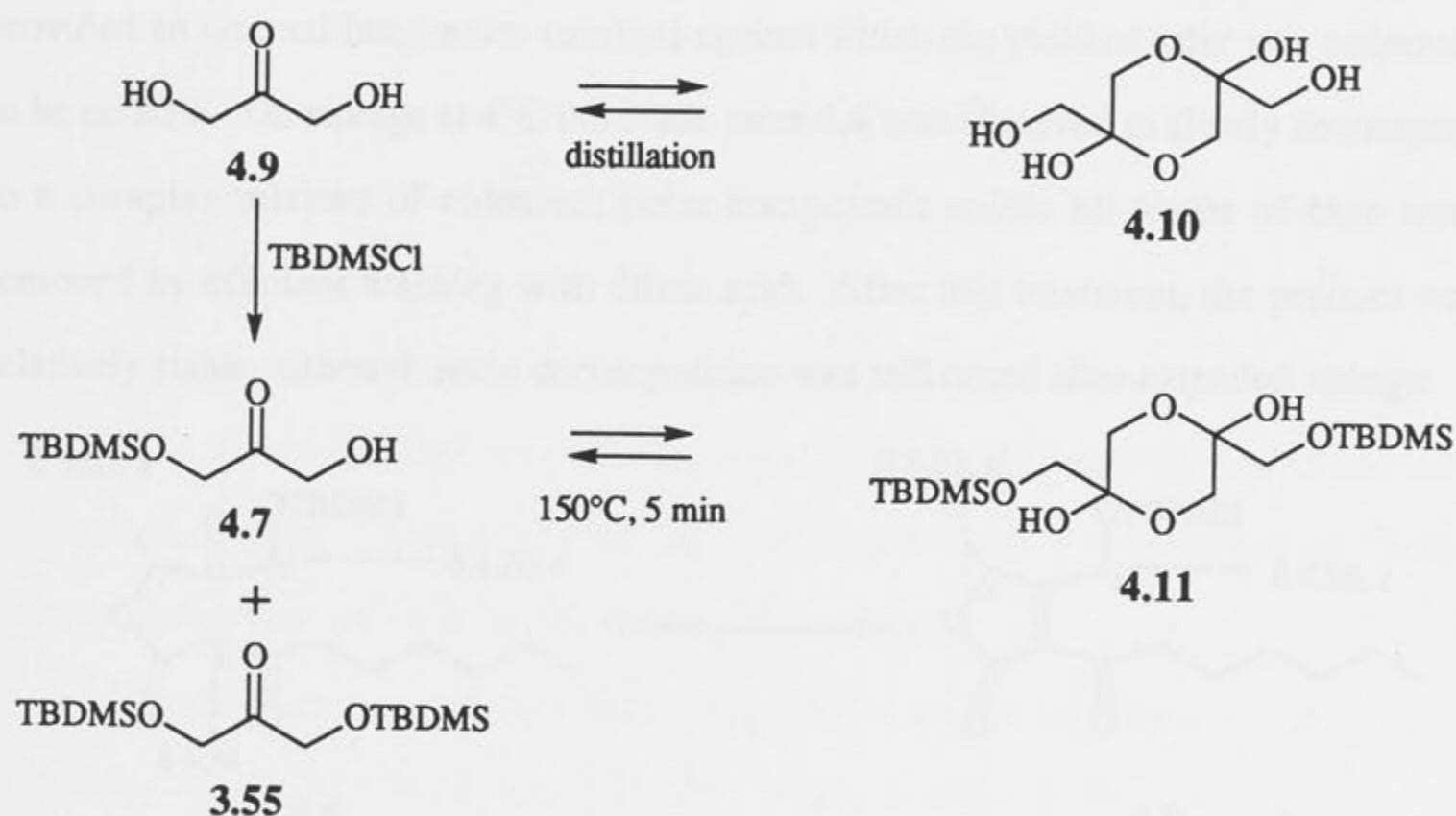
Scheme 4.4

### 4.2.1 Preparation and Cyclisation of the $\beta$ -Ketoester **4.6**

Since the  $\beta$ -ketoacid **3.33c** was already in hand, we had only to prepare the monosilyl dihydroxyacetone **4.7**. Dihydroxyacetone (**4.9**) is produced commercially by the microbial oxidation of glycerol,<sup>140</sup> but it readily dimerises to the 1,4-dioxane

**4.10**,<sup>141</sup> in which form it is marketed. The monomer can be reformed by distillation of the dimer;<sup>142</sup> higher polymers have been reported to form upon extended storage.<sup>143</sup>

Monomeric dihydroxyacetone was protected by reaction with *t*-butyldimethylsilyl chloride (TBDMS chloride) in the presence of triethylamine and 4-dimethylaminopyridine (DMAP) (Scheme 4.5). The use of an excess (2.5 fold) of the inexpensive dihydroxyacetone (**4.9**) ensured that only a small amount (3%) of the disilylated product **3.55** was formed and the monoprotected dihydroxyacetone **4.7** was isolated in 68% yield after distillation (based on TBDMS chloride).



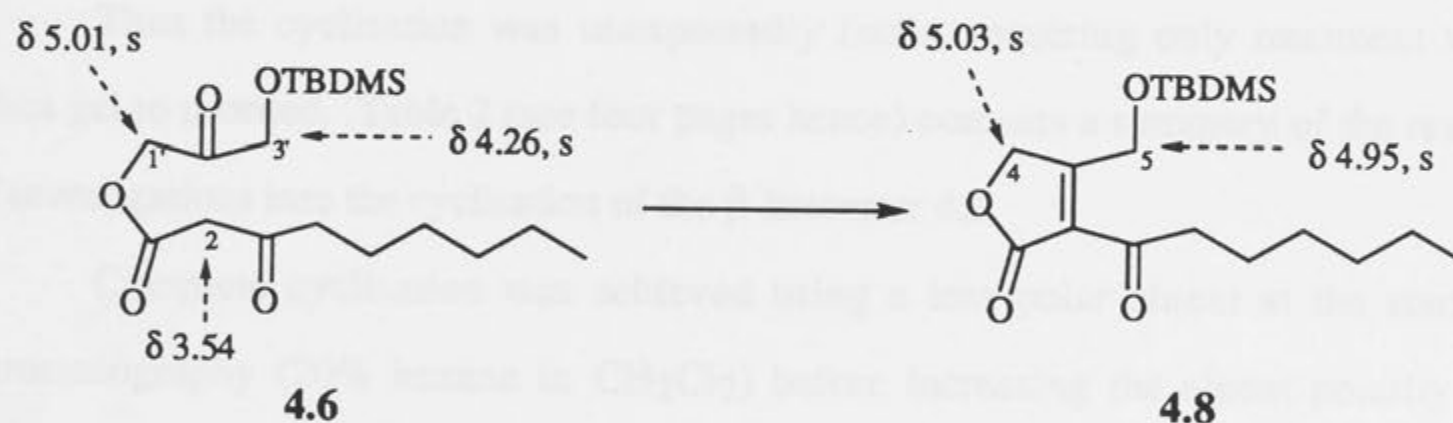
Scheme 4.5

Following fractional distillation, the monosilyl dihydroxyacetone **4.7** was also observed to slowly dimerise, a process found to be accelerated by the presence of acidic impurities. While the methylene resonances of the monomer appeared as sharp downfield singlets ( $\delta$  4.30 and  $\delta$  4.49), those of the dimer appeared as a complex system of peaks ( $\delta$  3.40-4.25) due to the geminal couplings and the presence of diastereomers in this cyclic form. As with the parent compound **4.9**, the silylated monomer **4.7** was found to be regenerated by distillation or brief heating (5 min., 150°C) of the dimer **4.11**. Silica gel chromatography was also found to promote this reversion. The direct, selective protection of the primary hydroxyl groups of the commercial



dihydroxyacetone 'dimer' **4.10** was not feasible due to its inhomogeneity and poor solubility, probably indicating the presence of higher polymers.<sup>143</sup>

Esterification of 3-oxononanoic acid (**3.33c**) with the monosilyl dihydroxyacetone **4.7** was accomplished using *N,N*-dicyclohexylcarbodiimide (DCC) catalysed by 4-dimethylaminopyridine<sup>123</sup> in  $\text{CH}_2\text{Cl}_2$ . Analysis of the crude product mixture by  $^1\text{H}$  NMR spectroscopy allowed identification of the *O*-silyl  $\beta$ -ketoester **4.6** as the major component by its three distinctive down-field methylene singlets, as shown in Scheme 4.6. The addition to the NMR tube of a known quantity of diphenylmethanol provided an internal integration standard against which the yield of ester was estimated to be *ca* 80%. On storage at  $4^\circ\text{C}$  the crude ester **4.6** was observed to slowly decompose to a complex mixture of coloured, polar compounds unless all traces of base were removed by efficient washing with dilute acid. After this treatment, the product was relatively stable, although some decomposition was still noted after extended storage.



Scheme 4.6

Attempted purification of the crude silylated  $\beta$ -ketoester **4.6** by radial chromatography ( $\text{SiO}_2$ ,  $\text{CH}_2\text{Cl}_2$ ) led to the recovery of only 12% of the product **4.6** and 50% of a new compound with a slightly higher  $R_f$  value. The  $^1\text{H}$  NMR spectrum of the new compound lacked the characteristic  $\beta$ -ketoester C2 methylene resonance near  $\delta$  3.5. However, it displayed two distinctive, closely-spaced downfield methylene singlets ( $\delta$  5.03 and 4.95) in addition to the signals expected for a heptanoyl substituent and a TBDMS group. The  $^{13}\text{C}$  NMR spectrum contained one ketone carbonyl resonance at  $\delta$  197.1 and three other low-field quaternary peaks ( $\delta$  181.1, 170.6 and 122.4). Since the peak at  $\delta$  122.4 was likely to be one of a pair of olefinic carbon resonances,



the more proximate of the remaining downfield peaks ( $\delta$  170.6) was assigned as a very strongly deshielded olefinic carbon while the other peak ( $\delta$  181.1) was assigned as an ester or lactone carbonyl carbon. EIMS revealed a parent ion at  $m/z$  340 (HRMS indicated  $C_{18}H_{32}O_4Si$ ) with a major fragmentation at 283 ( $M^+ - tBu$ ). It became clear, to our surprise and delight, that we had directly obtained the unsaturated  $\gamma$ -lactone **4.8**, (Scheme 4.6). The infrared spectrum provided confirmation with the appearance of carbonyl stretching bands at 1775 and 1688  $cm^{-1}$ . As expected, these bands are shifted to lower frequencies than were observed for the saturated 2-acyl butyrolactone **3.40** (1780 and 1725  $cm^{-1}$ ) due to their conjugation with the olefin moiety.<sup>144</sup> An additional band at 1630  $cm^{-1}$  was assigned as the olefin stretching absorption. Ultraviolet absorbance maxima at 236 nm ( $\epsilon$  8100) and 314 nm ( $\epsilon$  2200) were consistent with the K ( $\pi-\pi^*$ ) and R-band ( $n-\pi^*$ ) absorbances, respectively, which are expected for a  $\beta,\beta$ -dialkyl- $\alpha,\beta$ -unsaturated ketone with an  $\alpha$ -exo double bond.<sup>103</sup>

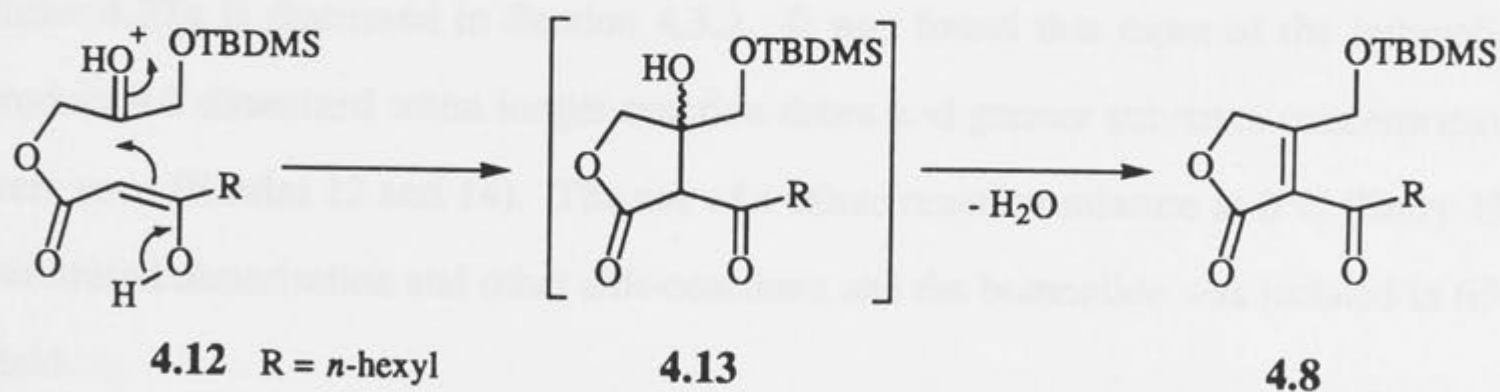
Thus the cyclisation was unexpectedly facile, requiring only treatment with silica gel to proceed. Table 2 (see four pages hence) contains a summary of the results of investigations into the cyclisation of the  $\beta$ -ketoester **4.6**.

Complete cyclisation was achieved using a less polar eluent at the start of chromatography (20% hexane in  $CH_2Cl_2$ ) before increasing the eluent polarity (to 100%  $CH_2Cl_2$ ; Entry 2). This allowed greater contact time between the substrate and silica gel and resulted in an increase in the yield of the butenolide **4.8** to 64% over the two steps. In an effort to perform the cyclisation independent of chromatography, the  $\beta$ -ketoester **4.6** was stirred with suspended silica gel (230-400 mesh, *ca* 20 mg  $SiO_2$  / 1 mg substrate) in  $CHCl_3$  (Entry 3). Only 30% cyclisation was observed after 3 days at room temperature, showing that the cyclisation apparently requires the more intimate contact with silica gel that chromatography provides. When the ester **4.6** was adsorbed onto silica gel in the absence of solvent (Entry 4), full cyclisation occurred within 20 minutes. Unfortunately, this treatment also caused extensive formation of polar by-products. Two dimensional TLC tests confirmed that the butenolide **4.8** decomposes to a mixture of low- $R_f$  compounds while adsorbed onto silica and, to a lesser extent,



during chromatography. For this reason, too much contact time between the substrate and silica gel is detrimental to yields. Column chromatography of the  $\beta$ -ketoester **4.6** (Entry 5) caused full cyclisation but gave a lower yield of butenolide **4.8**, presumably because the extra time that the silica was in contact with the substrate allowed more decomposition to occur. Similarly, it was found that further decreases in the polarity of eluent mixtures used in radial chromatography gave the butenolide in lower yield.

Silica gel is commonly regarded to be a mildly acidic medium and as such has been used to promote reactions which only proceed *via* acid catalysis such as the hydrolysis of acetals.<sup>145</sup> It is conceivable that the cyclisation of  $\beta$ -ketoester **4.6** also proceeds *via* an acid catalysed process, rather than through the enolate as previously discussed. The enol form of the protonated ketone **4.12** could cyclise to form lactone **4.13** and dehydrate to the butenolide **4.8** as depicted in Scheme 4.7. This cyclisation is a stereoelectronically 'favoured' 5-(*ENOLEXO*)-*EXO-TET* process. The proposition that the surprising ease of cyclisation was due to acid catalysis was tested in a series of cyclisation tests carried out under a variety of acidic conditions (Table 4.2, Entries 6 - 10).



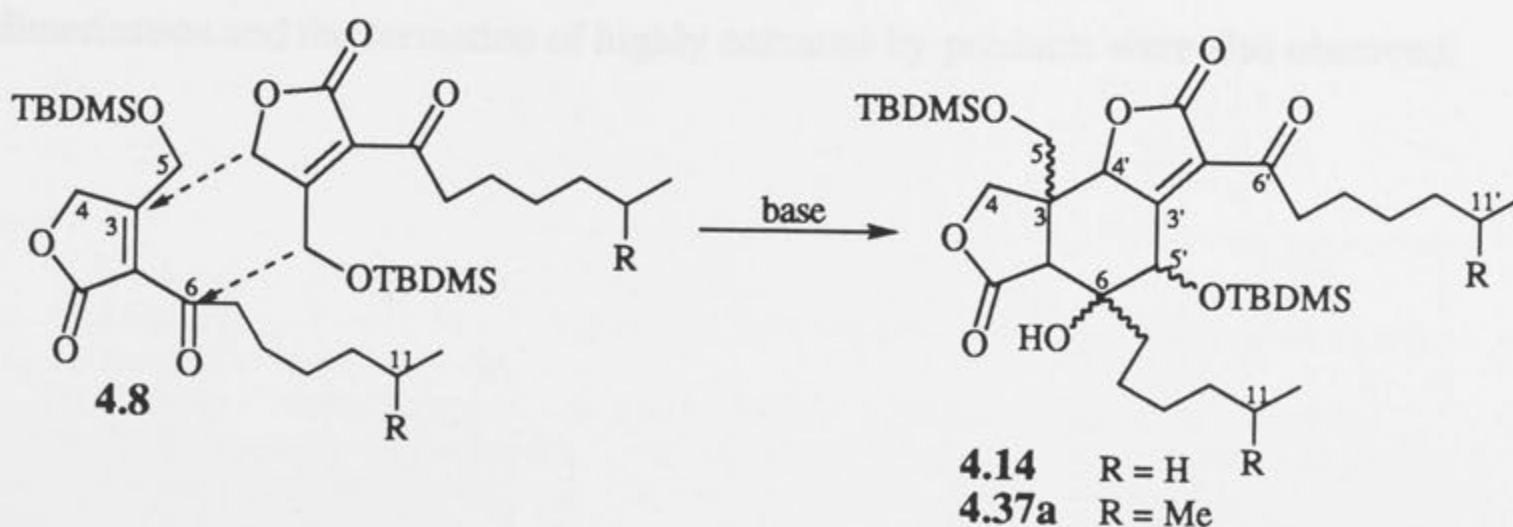
Scheme 4.7

Stirring the cyclisation precursor **4.6** in a suspension of acid-buffered silica gel (pH 4.5) (Entry 6), gave no significant increase in the rate of cyclisation over unbuffered silica gel. After treatment of ester **4.6** with  $d_4$ -acetic acid in  $CDCl_3$  for one hour at room temperature (Entry 7), only deuterium exchange was observed by  $^1H$  NMR spectroscopy. The presence of *p*-toluenesulfonic acid (*p*TSA, Entry 8) with the substrate in  $CDCl_3$  also failed to catalyse any cyclisation after 1 hour. Attempts to promote cyclisation with Lewis acids were also unsuccessful. Treatment with titanium



tetrachloride / pyridine<sup>146</sup> (Entry 9) gave only starting material after 5 hours at room temperature. Anhydrous zinc chloride in ether (Entry 10) also failed to induce any cyclisation. Thus, it appears unlikely that the cyclisation proceeds *via* an acid catalysed mechanism.

In contrast, base catalysis resulted in rapid cyclisation. Formation of the sodium enolate of the  $\beta$ -ketoester **4.6** with 1.1 equivalents of sodium hydride in ether resulted in complete cyclisation within 10 minutes at room temperature (Entry 11). The butenolide **4.8** was isolated as a yellow oil after rapid radial chromatography ( $\text{CH}_2\text{Cl}_2$ ) in a combined yield of 58%. Next eluted was a minor component which was isolated as colourless prisms (mp 130-1°C) and identified as the tricyclic dimer **4.14** (4%). The rest of the product appeared as a mixture of red-brown polar compounds whose complexity did not encourage further examination. Identification of the minor crystalline by-product **4.14** was eventually made by  $^1\text{H}$  NMR comparison with the closely related 11,11'-dimethyl compound **4.37a** which was formed in a directly analogous reaction during the subsequent synthesis of A-factor (**1.1**). The formation of the dimer by a base-catalysed annelation (Scheme 4.8) and structural assignment of dimer **4.37a** is discussed in Section 4.3.2. It was found that more of the butenolide product **4.8** dimerised when longer reaction times and greater substrate concentrations were used (Entries 12 and 14). The use of a dilute reaction mixture at 0°C (Entry 13), minimised dimerisation and other side-reactions and the butenolide was isolated in 65% yield.



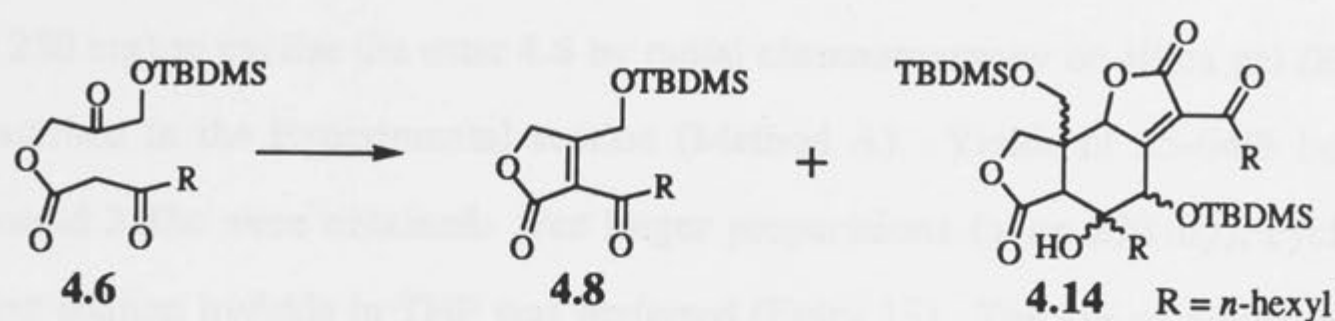
Scheme 4.8



Amine bases (diisopropylamine or triethylamine) also promoted cyclisation but did not prevent dimer formation despite their lower basicity. Cyclisation experiments were conveniently performed on a small scale in NMR tubes, allowing direct monitoring of the reaction by  $^1\text{H}$  NMR spectroscopy. It was found that the addition of 1.1 equivalents of either amine to a  $\text{CDCl}_3$  solution of the cyclisation precursor **4.6** resulted in a slower cyclisation than was observed using sodium hydride as base, requiring *ca* 30 minutes for completion. However, during this period approximately 10% of the product dimerised (Entries 15 and 17). The weaker base pyridine failed to promote any cyclisation even when large excesses were used (Entry 18).

The cyclisation was also found to proceed with non-stoichiometric quantities of base. The addition of 0.5 equivalents of diisopropylamine promoted the cyclisation of 80% of a sample of ester **4.6** after 30 minutes at room temperature in  $\text{CDCl}_3$ , during which time approximately 10% of this product dimerised (Entry 16). The presence of 0.3 equivalents of sodium hydride in ether induced full cyclisation but also extensive dimer formation (30%) after 20 minutes at room temperature (Entry 14). A catalytic quantity (0.02 eq.) of DMAP was found to catalyse the cyclisation of 50% of a sample of ester **4.6** in  $\text{CDCl}_3$  over an 8 day period at room temperature, with 10% concurrent dimerisation and the formation of highly coloured polar by-products (Entry 19). This observation confirms the need to remove all traces of DMAP from the  $\beta$ -ketoester **4.6** after esterification. Full cyclisation could also be catalysed by suspended potassium carbonate (Entry 20), with reaction being promoted by agitation. However, extensive dimerisation and the formation of highly coloured by-products were also observed.



Table 4.2 Cyclisations of  $\beta$ -Ketoester (4.6)

Entry No.	Reagent, Solvent	Time, Temp.	Ratio % ii	Yield v
			4.6 : 4.8 : 4.14	
1	SiO <sub>2</sub> radial chromatography, CH <sub>2</sub> Cl <sub>2</sub>	10 min., r.t.	20 : 80 : 0 iii	50%
2	SiO <sub>2</sub> radial chromatography, 20% hexane in CH <sub>2</sub> Cl <sub>2</sub> to CH <sub>2</sub> Cl <sub>2</sub>	12 min., r.t.	0 : 100 : 0	64%
3	SiO <sub>2</sub> , CHCl <sub>3</sub> (suspension)	72 h, r.t.	70 : 30 : 0	22%
4	SiO <sub>2</sub> , solvent-free	20 min., r.t.	0 : 100 : 0 iv	30%
5	SiO <sub>2</sub> Column chromatography, 20% hexane in CH <sub>2</sub> Cl <sub>2</sub>	20 min., r.t.	0 : 100 : 0 iv	36%
6	SiO <sub>2</sub> (pH 4.5), CHCl <sub>3</sub> (suspension)	72 h, r.t.	73 : 27 : 0	20%
7	d <sub>4</sub> -acetic acid (xs), CDCl <sub>3</sub>	1 h, r.t.	100 : 0 : 0	i
8	<i>p</i> -TSA (1 eq.), CDCl <sub>3</sub>	1 h, r.t.	100 : 0 : 0	i
9	TiCl <sub>4</sub> (2.0 eq.), pyridine, CCl <sub>4</sub> , THF	5 h, r.t.	100 : 0 : 0	0%
10	ZnCl <sub>2</sub> (anhydrous, 5 eq.), ether	1.5 h, r.t.	100 : 0 : 0	0%
11	NaH (1.1 eq.), ether (10 mg/mL)	10 min., r.t.	0 : 93 : 7	58%
12	NaH (1.1 eq.), THF (40 mg/mL)	20 min., r.t.	0 : 70 : 30 iv	35%
13	NaH (1.1 eq.), THF (5 mg/mL)	40 min., 0°C	0 : 96 : 4 iii	65%
14	NaH (0.3 eq.), ether (20 mg/mL)	20 min., r.t.	0 : 70 : 30	—
15	<i>i</i> -Pr <sub>2</sub> NH (1.1 eq.), CDCl <sub>3</sub>	30 min., r.t.	0 : 90 : 10	i
16	<i>i</i> -Pr <sub>2</sub> NH (0.5 eq.), CDCl <sub>3</sub>	30 min., r.t.	20 : 70 : 10	i
17	Et <sub>3</sub> N (1.1 eq.), CDCl <sub>3</sub>	30 min., r.t.	0 : 90 : 10	i
18	pyridine (5 eq.), CDCl <sub>3</sub>	2 h, r.t.	100 : 0 : 0	i
19	DMAP (0.02 eq.), CDCl <sub>3</sub>	8 days, r.t.	50 : 40 : 10	i
20	K <sub>2</sub> CO <sub>3</sub> (xs), CDCl <sub>3</sub> (suspension)	2 h, r.t.	0 : 75 : 25 iv	i

i) NMR tube experiment; products not isolated.

ii) Ratios obtained by <sup>1</sup>H NMR integration of the crude reaction mixture unless otherwise stated.

iii) Ratio of isolated compounds.

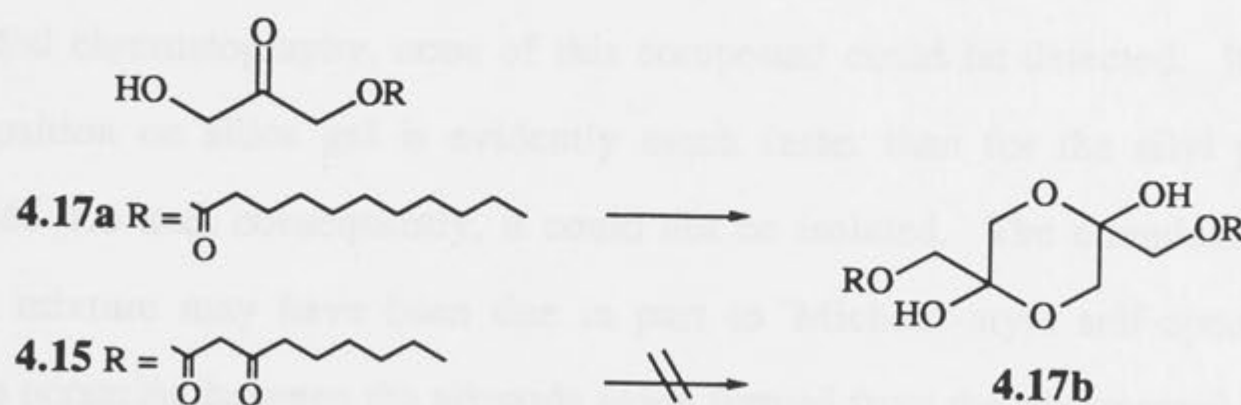
iv) Reaction mixture also contains significant amounts of unidentified polar by-products.

v) Yield for combined esterification and cyclisation steps based on 3-oxononanoic acid (3.33c).



In summary, it was found to be most convenient for small scale preparations (< *ca* 250 mg) to cyclise the ester **4.6** by radial chromatography on silica gel (Entry 2) as described in the Experimental section (Method A). Yields of 55-64% based on  $\beta$ -ketoacid **3.33c** were obtained. For larger preparations (> *ca* 250 mg), cyclisation utilising sodium hydride in THF was preferred (Entry 13). The use of dilute solutions (*ca* 5 mg/mL) minimised dimerisation of the product, while a low reaction temperature (0°C) minimised side reactions and allowed time for monitoring of the reaction by reverse phase TLC. Cyclisation does not occur to a significant extent during reverse phase chromatography. After cyclisation is complete (*ca* 40 min.), work-up and rapid radial chromatography (SiO<sub>2</sub>, 100% CH<sub>2</sub>Cl<sub>2</sub>) gave the butenolide in 55-65% yield over the both steps, in addition to *ca* 3% of the more polar dimer **4.14**.

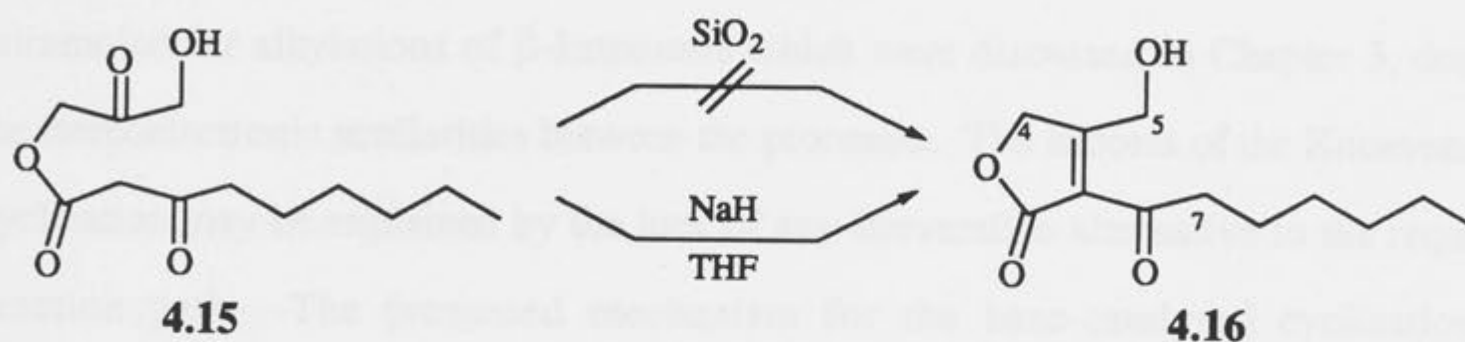
Attempts were made to perform the analogous cyclisation on the unprotected  $\beta$ -ketoester substrate **4.15** in an effort to avoid silyl protection. The ester **4.15** was prepared in 50% yield by esterification of 3-oxononanoic acid (**3.33c**) with an excess of dihydroxyacetone (3 eq.) using DCC / DMAP. The product was in this case a solid which was recrystallised from ether / hexane as colourless platelets (mp 68-70°C). The appearance of two ketone carbonyl peaks ( $\delta$  202.3, 203.8) in its <sup>13</sup>C NMR spectrum clearly showed the compound to be monomeric despite the report by Garson and co-workers<sup>147</sup> that another monoester of dihydroxyacetone (1-undecanoyl-3-hydroxyacetone, **4.17a**) readily dimerised to the 1,4-dioxane **4.17b**.



Scheme 4.9 Dimerisation of Dihydroxyacetone Derivatives



After either radial chromatography of **4.15** on silica gel or adsorption onto dry silica gel, no sign of the characteristic low field pair of methylene singlets near  $\delta$  5.0 which are expected for butenolide **4.16** was observed by  $^1\text{H}$  NMR spectroscopy. In both cases the majority of the ester **4.15** was recovered (*ca* 60%), but it was accompanied by complex unidentified polar compounds.



Scheme 4.10

When the unprotected ester **4.15** was subjected to sodium hydride promoted cyclisation conditions identical to those successfully employed for the silyl ester **4.6** (Table 4.2, Entry 11), a complex mixture of products resulted. The  $^1\text{H}$  NMR spectrum of the crude reaction mixture showed a product, representing an estimated 10-15% of the material, which was tentatively identified as the hydroxy butenolide **4.16**. It displayed two prominent singlet peaks at  $\delta$  4.94 and  $\delta$  5.01, which are very close to the C5 and C4 methylene resonances observed for the silylated butenolide **4.8** ( $\delta$  4.95 and  $\delta$  5.03). In addition, a triplet appearing at  $\delta$  2.98 ( $J = 7$  Hz) was assigned as the H7 methylene protons of the butenolide **4.16**, since the chemical shift and coupling constant were also very close to those displayed by the silyl butenolide **4.8**. However, after radial chromatography, none of this compound could be detected. Its rate of decomposition on silica gel is evidently much faster than for the silyl protected butenolide **4.6** and, consequently, it could not be isolated. The complexity of the reaction mixture may have been due in part to 'Michael'-style self-condensation reactions occurring between the alkoxide anion formed from the unprotected hydroxyl group of the butenolide **4.16** and the highly activated olefin moiety. Thus protection of the hydroxyl group proved to be necessary to ensure a 'cleaner' cyclisation reaction and a much more stable butenolide intermediate.







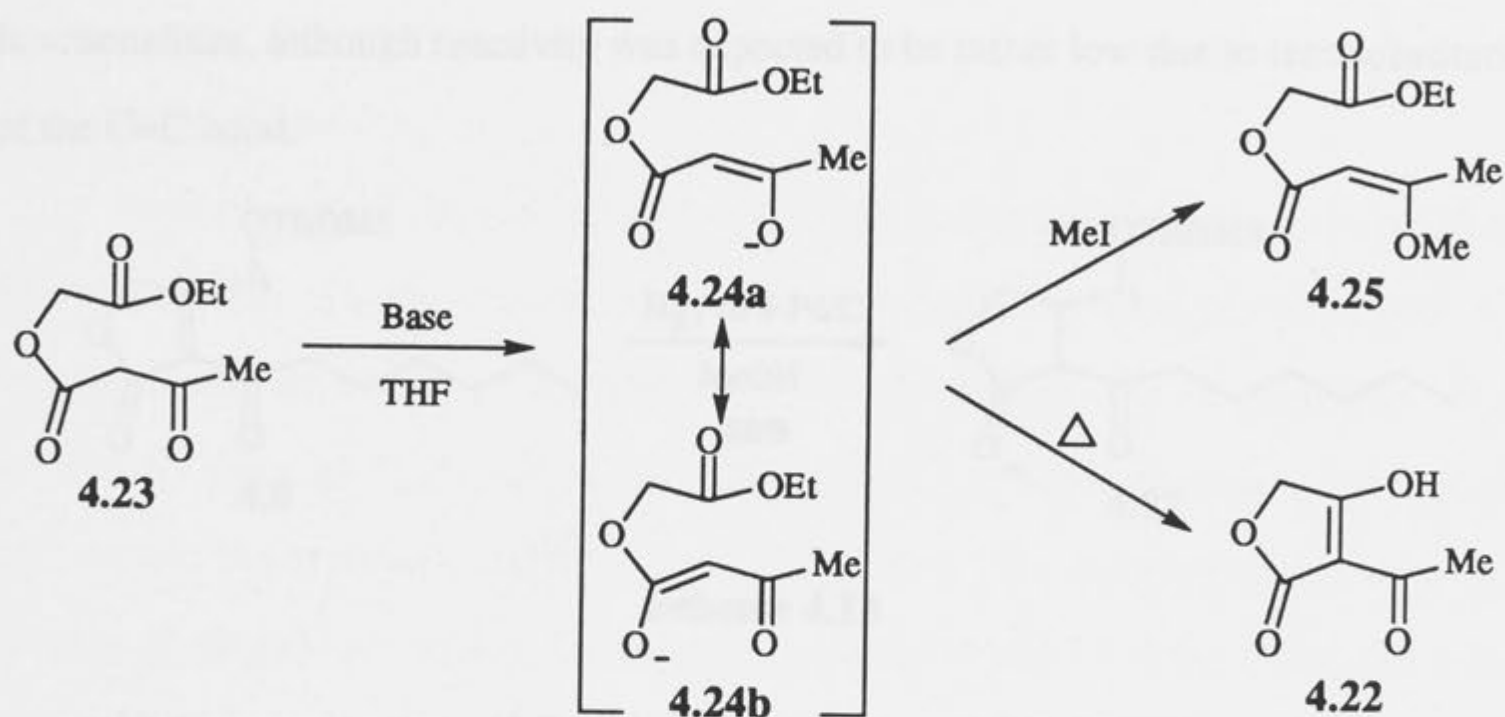
Cyclisation through the  $\beta$ -ketoenolate oxygen (4.18, path A) is a stereoelectronically 'favoured' 5-*EXO-TRIG* process which may be expected to compete with the desired mode of cyclisation (path B) as observed in the previously explored intramolecular alkylations (Chapter 3). However, in this case, oxygen attack leads to the unstable alkoxy-ketene acetal 4.19, which would readily revert to the stabilised enolate 4.18. Attack by the enolate carbon (path B), although stereoelectronically disfavoured, leads eventually to the thermodynamically stable butenolide 4.8. The initial attack to form the alkoxy-lactone 4.20 is also readily reversible, but this alkoxide can undergo a proton transfer to generate the resonance-stabilised 3-hydroxy  $\gamma$ -lactone enolate 4.21. Hydroxide ion may then be eliminated to form the butenolide 4.8, a process involving a favourable increase in entropy. The released hydroxide may then deprotonate more starting material and thus complete the catalytic cycle, as depicted in Scheme 4.11.

In essence, the readily reversible nature of the two possible cyclisations involving nucleophilic addition to the 2'-carbonyl group puts the outcome of the reaction under thermodynamic control. The stereoelectronic analyses of various cyclisations described by Baldwin<sup>109-111</sup> only predict the relative *rates* of these processes by assessment of the difficulty involved in achieving the required transition-state geometry. Nothing is implied about the relative thermodynamic stabilities of the compounds produced. Thus it is possible to observe apparent contravention of these 'rules' in the case of thermodynamically controlled reactions if a slower, 'disfavoured' process leads to a more stable product than a competing 'favoured' process.

Ager and Mole, however, attempt to rationalise the success of a similar type of cyclisation (see Entry 1c, Table 4.1) using stereoelectronic arguments alone.<sup>137</sup> The acyl tetronic acid 4.22 is produced *via* the intramolecular condensation (Dieckmann condensation) of the  $\beta$ -ketoester 4.23. They postulate that the reaction succeeds because it is able to utilise the stereoelectronically 'favoured' 5-(*ENOLEXO*)-*EXO-TRIG* pathway due to a supposed preference for the *ENOL-EXO* form of the enolate 4.24a.

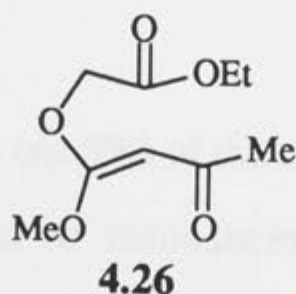


Their argument is based on the result of a trapping experiment in which the lithium enolate **4.24** in THF was treated with an excess of methyl iodide to give a 75% yield of a product assigned as the *O*-methyl enol-ether **4.25** (Scheme 4.12), although no evidence supporting this structure was reported.



Scheme 4.12

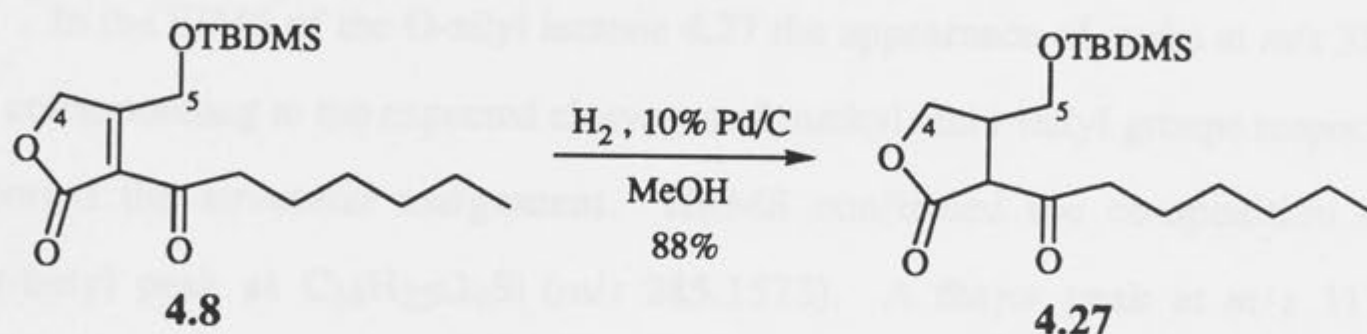
Both this result and their mechanistic supposition are likely to be in error. Other studies of the alkylation of  $\beta$ -ketoesters with alkyl iodides clearly show a strong preference for C- rather than O-alkylation products.<sup>149</sup> For example, in their study of C- vs. O-alkylation, Guibe *et al.*<sup>150</sup> show that alkylation of the lithium enolate of methyl acetoacetate with ethyl iodide in dimethoxyethane gives a C : O alkylation ratio of >100:1. In the previous chapter (see Scheme 3.19), it was described how the trapping with methyl iodide of the sodium enolate of methyl 3-oxononanoate (**3.33a**) in THF gave a product which was unambiguously identified by <sup>1</sup>H NMR as the C-methyl compound **3.54**, in 80% yield. Even if the *O*-methyl enol-ether **4.25** has been isolated it would not demonstrate any preference for either canonical form. It could simply demonstrate greater thermodynamic stability of this product over the alternative O-methylation product, which is the unstable ketene acetal **4.26**.





## 4.2.3 Reduction of the Butenolide 4.8

Catalytic hydrogenation was the method chosen as most likely to achieve the reduction of the tetrasubstituted olefin **4.8** without affecting the silyl, keto or ester functionalities, although reactivity was expected to be rather low due to tetrasubstitution of the C=C bond.



Scheme 4.13

Hydrogenation over 10% palladium-on-carbon (10% Pd/C) in methanol at room temperature and pressure was found to proceed surprisingly rapidly. One equivalent of hydrogen was consumed after only 40 minutes, by which time hydrogen uptake had virtually ceased. After removal of the catalyst, the saturated lactone **4.27** was isolated as an oil in 88% yield (Scheme 4.11). As expected, the  $^1\text{H}$  NMR spectrum of the product was considerably more complex than the starting material due to the additional coupling with the two new protons. The proton at C2 appeared as a doublet ( $\delta$  3.64,  $J_{2,3} = 6.8$  Hz) and the proton at C3 gave a multiplet ( $\delta$  3.18) due to coupling with five vicinal protons. The C4 methylene protons both appeared as down-field AB quartets ( $\delta$  4.39 and  $\delta$  4.12) while the two C5 protons were almost co-incident, appearing as a pseudo-doublet at higher field ( $\delta$  3.62). In the  $^{13}\text{C}$  NMR spectrum, saturation of the olefin moiety was confirmed by the disappearance of the quaternary resonances and the appearance of two methine peaks at  $\delta$  54.7 and  $\delta$  39.3, assigned to C2 and C3 respectively. Methylene peaks at  $\delta$  69.1 and  $\delta$  61.9 were assigned to C4 and C5 respectively.<sup>103</sup>

Apart from a small amount (*ca* 5%) of the enol form identified by the enolic OH at 11.2, no sign of a second set of resonances was noted by  $^1\text{H}$  or  $^{13}\text{C}$  NMR

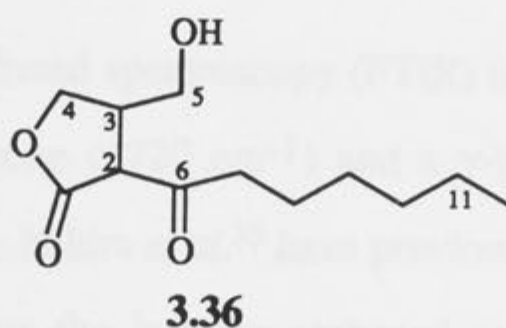


spectroscopy. This suggested that the product contains a single diastereomer which was presumed to be the 2,3-*trans* diastereomer since the lower steric interaction between the substituents in this form was expected to make it thermodynamically more stable. Epimerisation at C2 *via* the enol is presumably facile. The question of C2,3 relative stereochemistry is addressed more fully with reference to the corresponding 11-methyl compound 4.35, see Section 4.2.3.

In the EIMS of the O-silyl lactone 4.27 the appearance of peaks at  $m/z$  327 and 285, corresponding to the expected cleavages of methyl and *t*-butyl groups respectively, supported the structural assignment. HRMS confirmed the composition of the  $M^+ - t\text{-butyl}$  peak as  $C_{14}H_{25}O_4Si$  ( $m/z$  285.1523). A major peak at  $m/z$  113 was attributed to the heptanoyl fragment ion ( $C_6H_{13}CO^+$ ). The infrared spectrum in  $CCl_4$  solution displayed two carbonyl stretching bands (1779 and  $1720\text{ cm}^{-1}$ ) corresponding to typical  $\gamma$ -lactone and aliphatic ketone carbonyls respectively.

It was found that prolonged hydrogenation (4 h, MeOH, 10% Pd/C) resulted in the partial cleavage of the TBDMS protecting group. Hydrogenolysis of the TBDMS moiety was unexpected<sup>151,152</sup> but not unprecedented,<sup>153</sup> although it is perhaps more likely that the TBDMS group was removed by methanolysis catalysed by the traces of hydrochloric acid which are generally present in Pd/C.<sup>154</sup> A waxy solid was isolated in 15% yield which had a very similar  $^1H$  NMR spectrum to the silylated lactone 4.27, except that there were no signals corresponding to a TBDMS group and a broad OH peak at  $\delta$  1.8 was present which readily exchanged on  $D_2O$  addition. The identification of this product as the desired 11-demethyl A-factor (3.36) was supported by the appearance of a molecular ion at  $m/z$  228 in the EI mass spectrum. The low yield and the presence of a substantial amount (*ca* 10%) of an unwanted by-product in the reaction mixture made this method unattractive as a preparative, 'one pot' route to the desilylated product.





#### 4.2.4 Desilylation to 11-Demethyl A-Factor (3.36)

The *t*-butyldimethylsilyl (TBDMS) protecting group is generally stable under basic or neutral conditions but may be cleaved easily by treatment with acid (*e.g.* dilute acetic acid) or with the use of fluoride.<sup>152</sup> Deprotection of the TBDMS-lactone **4.27** under acid conditions was found to be rather slow. After treatment with acetic acid / THF / water (3 : 3 : 1), as recommended by Corey and coworkers,<sup>152</sup> approximately 15% of unreacted protected lactone **4.27** was isolated in addition to 55% of 11-demethyl A-factor **3.36** after 48 hours at room temperature. Attempts to improve the yield using longer reaction times were complicated by the concurrent increase in the formation of several by-products, as observed by TLC. Treatment of the silyl lactone **4.27** with tetra-*n*-butylammonium fluoride (THF, 3 eq.) for one hour at room temperature gave the product in an improved yield (80%), but the concurrent formation of several polar by-products indicated that a milder desilylation method was required. A mixture of aqueous hydrofluoric acid and pyridine in CH<sub>3</sub>CN has been reported as a mild and effective reagent for the removal of TBDMS groups from sensitive prostaglandin substrates.<sup>155</sup> Using this method the TBDMS group was smoothly removed in 7 hours at room temperature to give 11-demethyl A-factor **3.36** in 90% yield as a colourless oil which solidified to a waxy solid upon cooling. The identity of the product was supported by high resolution mass spectroscopy of its molecular ion, which indicated a composition of C<sub>12</sub>H<sub>20</sub>O<sub>4</sub>, as required. Fragment ions in the EIMS confirmed the substituents on the lactone ring. A peak assigned to the loss of the C3 hydroxymethyl fragment (*m/z* 197, M<sup>+</sup>-CH<sub>2</sub>OH, 16%) was present, as was a heptanoyl fragment ion (*m/z* 113, C<sub>7</sub>H<sub>13</sub>O<sup>+</sup>, 68%).



Fourier transform infrared spectroscopy (FTIR) in  $\text{CCl}_4$  solution confirmed the presence of a saturated ketone ( $1720\text{ cm}^{-1}$ ) and a  $\gamma$ -lactone carbonyl ( $1785\text{ cm}^{-1}$ ; typically<sup>144</sup> near  $1780\text{ cm}^{-1}$ ). Nihira *et al.*<sup>35</sup> have previously synthesised this compound (see Scheme 1.7) but report the lactone carbonyl appearing at  $1760\text{ cm}^{-1}$  when measured as a thin film (solvent-free). This lower absorption frequency probably results from the increased intermolecular hydrogen bonding expected under these conditions. In all other respects, their IR data were consistent with those recorded here for lactone **3.36**. The reported  $^1\text{H}$  NMR data for this compound,<sup>35</sup> although recent, are of limited value since they have been acquired at only 60 MHz and include no assignments or coupling information. While the positions of the reported 'multiplets' are in general agreement with our 300 MHz  $^1\text{H}$  NMR data, the reported integration over two of these regions appears to be in error. The region  $\delta$  4.0-4.6 which encompasses the two doublets of doublets of the H4a and H4b protons, has been mistakenly reported as a 3 proton multiplet, and the system of peaks between  $\delta$  3.6 and  $\delta$  3.8 has been reported as a two-proton multiplet, when it actually contains the almost coincident signals of 3 protons (H2, H5a and H5b). These errors have been repeated in the data reported for eight other analogues prepared by these authors.<sup>35</sup> The spectrum of demethyl A-factor **3.36** was generally quite similar to that of the silyl precursor **4.27**, except that the H5 proton resonances were more widely separated giving two doublets of doublets. The vicinal  $J_{2,3}$  coupling constant was virtually unchanged at 7 Hz, suggesting that the 2,3-relative stereochemistry has been preserved.

The  $^{13}\text{C}$  spectrum of **3.36** was also very similar to that of the protected compound **4.27** except for the absence of signals due to the TBDMS group. The removal of the silyl group had surprisingly little effect on any of the resonances ( $< 1\text{ ppm}$ ). Although  $^{13}\text{C}$  NMR data for this compound have not been reported, the  $^{13}\text{C}$  data for A-factor itself (**1.1**),<sup>18</sup> although not assigned, provide a close model for the 3-hydroxymethyl  $\gamma$ -lactone nucleus. All the  $^{13}\text{C}$  resonances of A-factor, except those attributable to its methyl branched alkyl 'tail' (C8 to C12), corresponded closely (within 1 ppm) with a major peak in the spectrum of 11-demethyl A-factor **3.36**.



A puzzling feature of this compound was that in both the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra a co-occurring array of minor peaks (*ca* 10-20% peak heights) was observed which persisted even after further purification. These were eventually attributed to other tautomeric forms of the compound. The unexpected identity and significance of analogous components occurring with A-factor itself (**1.1**) are discussed in Chapter 5.

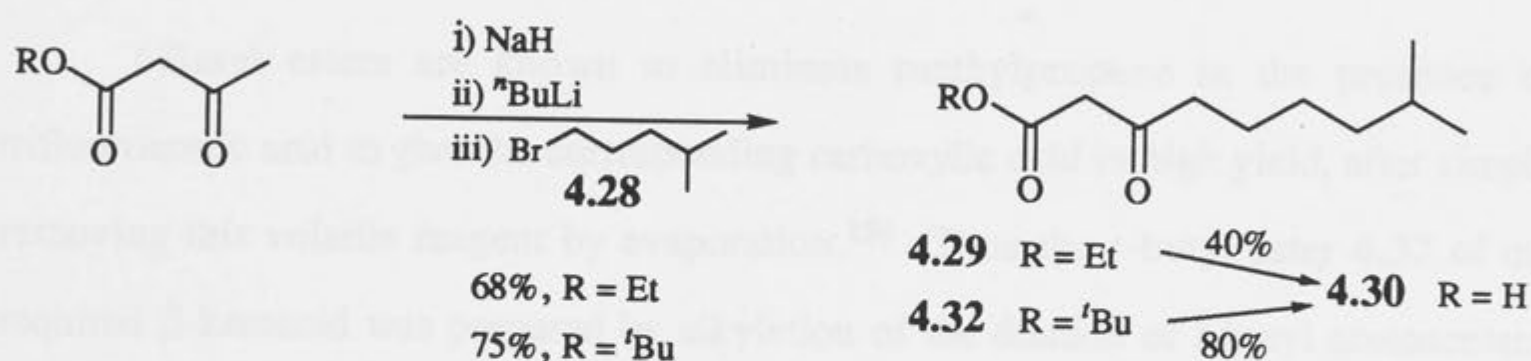
Thus 11-demethyl A-factor (**3.36**) has been successfully synthesised by an efficient biomimetic route, in an overall unoptimised yield of *ca* 20% from ethyl acetoacetate. Already, this represents a significant improvement over the existing synthesis of this compound in 4.5% yield from diethyl formylsuccinate (*via* a route analogous to Scheme 1.7, Chapter 1).<sup>35</sup> This synthesis demonstrates the viability of the biomimetic approach and opens the way for the development of an improved synthesis of A-factor itself (**1.1**). The biological activity of **3.36** has been reported to be less than 20% of that of A-factor itself (see Chapter 1, Table 1.1, Entry 5).<sup>4</sup>



### 4.3 THE BIOMIMETIC SYNTHESIS OF A-FACTOR

Adaptation of the preceding synthetic route into a synthesis of A-factor involves the incorporation of a terminal branched methyl substituent. The expense and relative difficulty in obtaining the required 1-bromo-4-methylpentane (**4.28**) provided incentive to improve the efficiency of the synthetic route, particularly in the early stages.

#### 4.3.1 Synthesis of 8-Methyl-3-Oxononanoic acid (**4.30**)



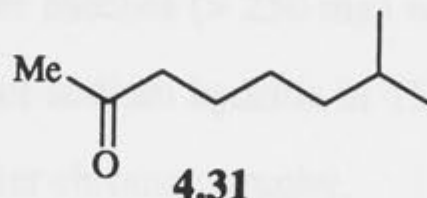
Scheme 4.14

Alkylation of the dianion of ethyl acetoacetate with commercially available 1-bromo-4-methylpentane (**4.28**, 1.1 eq.) gave the ethyl  $\beta$ -ketoester **4.29** in 62% yield after fractional distillation, a yield similar to that obtained for the preparation of the unbranched ethyl  $\beta$ -ketoester **3.33b**. A considerable quantity (29%) of the alkylating agent **4.28** was recovered unreacted while only a trace of unreacted ethyl acetoacetate was isolated. Monitoring of the reaction by gas chromatography confirmed that ethyl acetoacetate was consumed substantially faster than the alkylating agent, indicating that the yield of product is limited by side-reactions of the ethyl acetoacetate dianion.

Isolation of the  $\beta$ -ketoacid **4.30** after acid hydrolysis (AcOH / conc. HCl<sub>aq</sub>) of the ethyl ester **4.29** was more difficult than for the straight chain  $\beta$ -ketoacid due to the lower melting point of the product (mp 44-46°C vs 70-71°C) and its increased solubility in organic solvents. Recrystallisation of the crude product required cold pentane (-15°C) and it was necessary to collect and wash the crystals in a cold-room at 0°C to prevent melting or redissolution of the product. A yield of 40% of crystalline product



was obtained in three crops, and the mother liquor contained a further *ca* 20% of  $\beta$ -ketoacid which resisted crystallisation probably because of the substantial quantity of the decarboxylation product, the liquid methyl ketone **4.31**, which was also present. Non-crystalline, impure  $\beta$ -ketoacid was found to fully decarboxylate within a few days, even at 0°C. This disappointing yield of useable  $\beta$ -ketoacid prompted attempts to improve its synthesis.



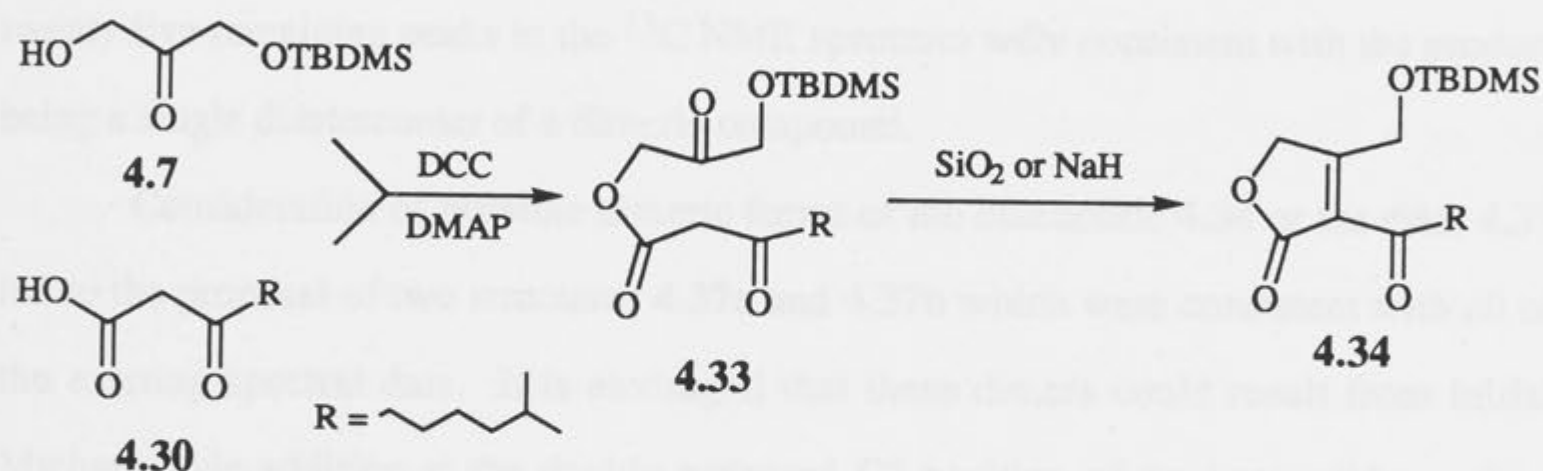
*t*-Butyl esters are known to eliminate methylpropene in the presence of trifluoroacetic acid to give the corresponding carboxylic acid in high yield, after simply removing this volatile reagent by evaporation.<sup>156</sup> Thus the *t*-butyl ester **4.32** of our required  $\beta$ -ketoacid was prepared by alkylation of the dianion of *t*-butyl acetoacetate, which is readily available and inexpensive (Scheme 4.14). By using a small excess of *t*-butyl acetoacetate (1.1 eq.) with respect to the 1-bromo-4-methylpentane (**4.28**), the side reactions which had been observed with the dianion of ethyl acetoacetate were avoided and the costly alkylating agent was fully consumed. The *t*-butyl  $\beta$ -ketoester **4.32** was isolated, after distillation, in a 75% yield based on the bromide **4.28**.

The *t*-butyl ester **4.32** was efficiently converted to the  $\beta$ -ketoacid **4.30** by treatment with an excess of trifluoroacetic acid in CH<sub>2</sub>Cl<sub>2</sub> for 2 hours at room temperature. Removal of the solvent under vacuum at room temperature gave the  $\beta$ -ketoacid **4.30** in an almost pure state. Recrystallisation from pentane at -15°C gave an 80% yield of fine colourless needles in two crops, with very little methyl ketone **4.31** being detected in the mother liquors. Samples of this product have proved stable towards decarboxylation for up to a year at 4°C.



### 4.3.2 Esterification and Cyclisation

Esterification of the  $\beta$ -ketoacid **4.30** with mono-protected dihydroxyacetone **4.7** and subsequent cyclisation were performed by the methods developed for the model synthesis (see Scheme 4.15). Esterification with DCC followed by cyclisation using silica gel chromatography as described above gave the unsaturated lactone **4.34** in 65% yield over the two steps. Larger batches (> 250 mg) were more conveniently cyclised by treatment with 1 equivalent of sodium hydride in THF (0°C), furnishing the product in slightly lower yield (60%) after chromatography.



Scheme 4.15

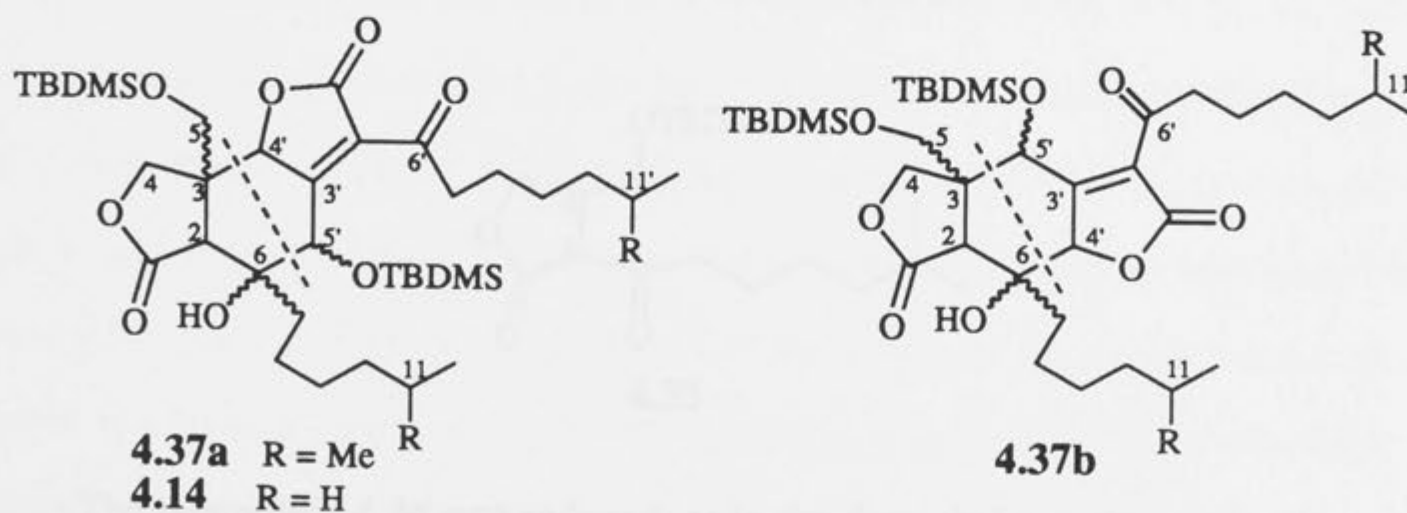
As observed in the cyclisation of the straight chain ester **4.6** with sodium hydride, a significant by-product was noted in the reaction mixture by TLC, especially when longer reaction times and/or more concentrated reaction mixtures were used. In some cases this compound represented 15-20% of the product mixture, separable by chromatography; it could be recrystallised as colourless needles from hexane, (mp 130-132°C). CIMS revealed a molecular weight of 708 for the compound, corresponding to twice the molecular weight of the silyl butenolide product **4.34**, indicating a dimeric structure. A distinctive feature of its <sup>1</sup>H NMR spectrum was a closely spaced pair of deshielded methine singlets at  $\delta$  5.38 and  $\delta$  5.34, which are similar in spacing to the H4 and H5 methylene singlets of the unsaturated lactone **4.34**, but are shifted downfield by *ca* 0.3 ppm. A broad peak at  $\delta$  2.0 which disappeared with the addition of D<sub>2</sub>O was assigned as an hydroxyl proton. Integration of the region



( $\delta$  1.5 to  $\delta$  -0.2) indicated the presence of two alkyl chains and two TBDMS groups giving further credence to a dimeric structure.

The  $^{13}\text{C}$  NMR spectrum of the dimer displayed five downfield quaternary resonances ( $\delta$  126.6, 169.2, 174.9, 176.3 and 197.7). The presence of only one ketone group was indicated by the single peak ( $\delta$  197.7) in this region. The resonance at  $\delta$  126.6 is close to the chemical shift of the C2 olefinic carbon of the butenolide product 4.34, which suggested that the dimeric compound may contain a similar  $\alpha$ -acyl- $\alpha,\beta$ -unsaturated lactone moiety. The remaining three quaternary peaks were therefore assigned as two lactone carbonyls and a strongly deshielded olefin. The twenty five remaining peaks in the  $^{13}\text{C}$  NMR spectrum were consistent with the product being a single diastereomer of a dimeric compound.

Consideration of possible dimeric forms of the butenolide 4.34 or the ester 4.33 led to the proposal of two structures 4.37a and 4.37b which were consistent with all of the existing spectral data. It is envisaged that these dimers could result from initial Michael-style addition at the doubly activated C3 position of the butenolide product 4.34 of another butenolide molecule which had been deprotonated at either C4 or C5. Annulation would be completed by base-promoted reaction between the remaining acidic site and the ketone carbonyl, resulting in a tricyclic dimer containing two  $\gamma$ -lactone moieties fused to create a central 6-membered ring with the molecule possessing either an angular (4.37a) or linear fused geometry (4.37b).



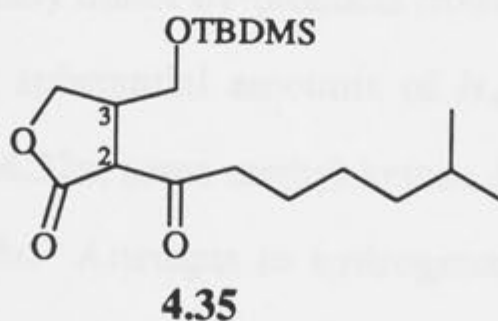
The appearance of a single set of peaks in the  $^{13}\text{C}$  NMR spectrum of the dimer shows the proposed annulation to be remarkably regio- and diastereoselective.



Evidently, only one of the regioisomers **4.37a** or **4.37b** is formed and a single relative configuration of its five asymmetric centres is preferred. Two dimensional  $^1\text{H}$ - $^1\text{H}$  (COSY) and  $^1\text{H}$ - $^{13}\text{C}$  (HETCOR) correlated spectra were obtained (see Appendices Ai and Aii) which supported our general structural proposal and facilitated assignment of the  $^1\text{H}$  and  $^{13}\text{C}$  spectra. However, the similarity of the expected NMR spectra for the two proposed regioisomeric structures **4.37a** and **4.37b** and the lack of any vicinal  $^1\text{H}$ - $^1\text{H}$  couplings between the two halves of these dimers made it difficult to distinguish between these two possibilities.

Close inspection of the 2D  $^1\text{H}$ - $^1\text{H}$  correlated spectrum (COSY, Appendix Ai) revealed several low-intensity cross-peaks representing small long-range couplings ( $< 0.5$  Hz) which were not apparent in the 1D  $^1\text{H}$  NMR spectrum, notably  $J_{2,5a}$ ,  $J_{2,5'}$ ,  $J_{4',5a}$ , and  $J_{4',4a}$ . While the first two are reconcilable with either dimeric structure **4.37a** or **4.37b** as four-bond ( $^4J$ ) couplings, the latter two ( $J_{4',5a}$ ,  $J_{4',4a}$ ) are only feasible for the angular fused-tricyclic dimer **4.37a**, since the protons involved are separated by too many bonds in the alternative, linear structure **4.37b**. Thus the angular structure **4.37a** is favoured and since the  $^1\text{H}$  NMR spectrum of the analogous demethyl dimer isolated in the previous reaction series (Section 4.1.3) was nearly identical, except for the resonances attributable to the alkyl chains, it too was assigned an angular fused-tricyclic structure **4.14**.

#### 4.3.3 TBDMS Protected A-Factor (4.35)



The butenolide **4.34** was reduced, as in the demethyl reaction series, by catalytic hydrogenation over 10% palladium-on-carbon. However, we found that ethyl acetate was a superior reaction solvent to methanol, since it does not promote the desilylation and other unwanted reactivity previously observed during extended hydrogenations



(Section 4.1.5). Using ethyl acetate and *ca* 3% by mass of catalyst, approximately 3 hours under ambient conditions were required for complete reduction of the butenolide **4.34** to TBDMS-protected A-factor **4.35**. No extraneous products were observed to appear by TLC even after 12 hours. The saturated lactone **4.35** was isolated as a colourless oil after chromatography, in 80-90% yield. So clean was the conversion that the variation in yield is more likely to reflect the purity of the butenolide than the efficiency of the reaction itself. It was found to be counterproductive to attempt to purify further the silyl butenolides **4.8** or **4.34** prior to hydrogenation in view of their instability, especially under basic conditions or in contact with silica gel. Additional purification of a sample of the butenolide **4.34** by further radial chromatography on silica gel (15% EtOAc in hexane) gave the compound of sufficient purity for satisfactory microanalysis but *ca* 40% of the sample was lost by decomposition. Attempted purification on alumina (PTLC) was found not only to be ineffective, but also to cause substantial decomposition to polar compounds. Sephadex chromatography (LH-20) at least did not cause degradation but was also ineffective for purification. Even when stored at 4°C under dry argon after efficient acid washing, the butenolide **4.34** underwent significant degradation to coloured polar material within a few weeks.

An obvious approach to the problem of instability of the butenolide **4.34** is to not purify it at all, but to catalytically hydrogenate the crude product. Unfortunately, the crude butenolide from sodium hydride promoted cyclisation was usually less than 50% pure and contains the many minor by-products from both the esterification and the cyclisation steps, including substantial amounts of *N,N*-dicyclohexylurea, probably some *N*-acyl urea, the dimer **4.37a**, some methyl ketone **4.31** and a veritable menagerie of complex polar compounds. Attempts to hydrogenate the crude butenolide **4.34** showed it to proceed very slowly (> 30 h), and required the addition of several extra portions of catalyst (total *ca* 6% by mass) to enable the reaction to go to completion. The crude butenolide **4.34** evidently contains an unidentified compound (or compounds) with the ability to interfere with catalytic hydrogenation, presumably by

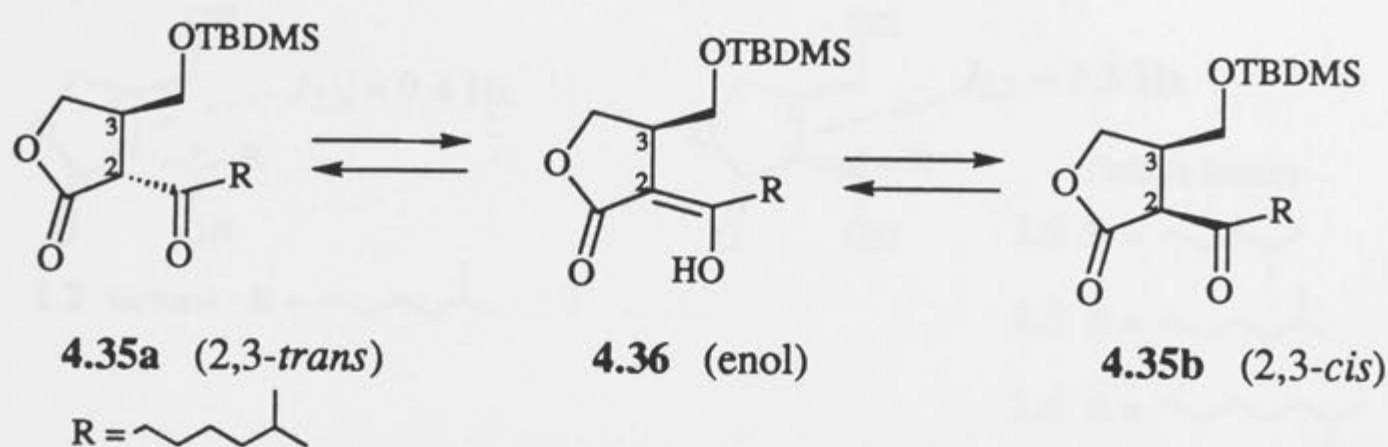


poisoning the catalyst. Extrication of the required silyl lactone **4.35** from the resulting complex reaction mixture was more difficult than usual due to the presence of close-running impurities and some of the product was inevitably found in mixed fractions which required further chromatography. The yield of O-silyl A-factor **4.35** from 3-oxononanoic acid is 40-55% which is less than that obtainable when the butenolide intermediate is purified (*ca* 58%). Thus the purification of the butenolide intermediate **4.34** by rapid radial chromatography appeared to be a prudent step which, although involving some loss, facilitates its efficient conversion to the A-factor precursor **4.35**.

TBDMS-protected A-factor **4.35**, in complete contrast with the preceding two intermediates, is a robust compound which is stable to silica gel chromatography, basic conditions and can even be purified by Kugelrohr distillation (oven temp. 130°C, 0.1 mm Hg). As such, it is a very suitable precursor since it can be readily purified or stored for long periods before deprotection to A-factor (**1.1**). Its purity and stability make it an excellent substrate for full NMR spectral characterisation, providing a well established benchmark for the eventual assignment of the spectra of A-factor (Chapter 5).

The  $^{13}\text{C}$  NMR spectrum is completely consistent with that of the previously synthesised 11-demethyl O-silyl A-factor **4.27**, apart from the expected differences involving the alkyl chain. All  $^{13}\text{C}$  and  $^1\text{H}$  NMR peaks were unambiguously assigned using two dimensional  $^1\text{H}$ - $^1\text{H}$  (COSY) and  $^1\text{H}$ - $^{13}\text{C}$  (HETCOR) correlated spectra (see Appendices Bi and Bii). In  $\text{CDCl}_3$  solution *ca* 5% of the product exists in its enol form **4.36**, as is clearly demonstrated by integration of the sharp, H-bonded enol hydroxy peak appearing at  $\delta$  11.2.

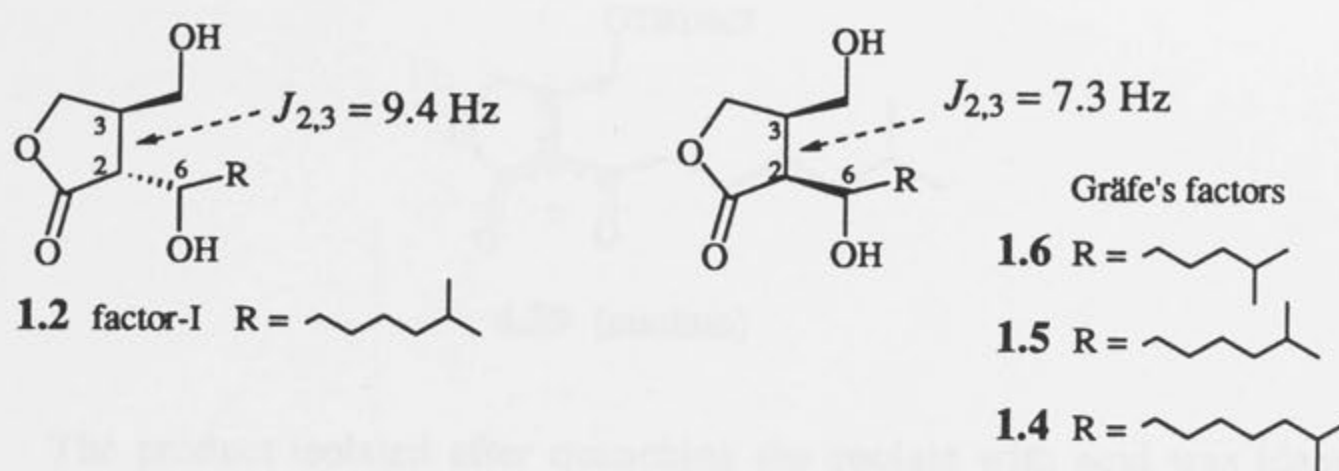




Scheme 4.16

As had previously been seen for the TBDMS-protected demethyl butanolide **4.27**, only one diastereomer of the keto form **4.35a** or **4.35b** was apparent in the  $^{13}\text{C}$  and  $^1\text{H}$  NMR spectra. Although it had been assumed that the 2,3-*trans* diastereomer would dominate the tautomeric equilibrium depicted in Scheme 4.16 due to its expected greater thermodynamic stability, the magnitude of the H2-H3 vicinal coupling constant ( $J_{2,3} = 6.8$  Hz) caused us to review this assumption. The magnitude of this coupling constant has been used by several groups<sup>26,28,35</sup> to assign the 2,3-relative stereochemistry of the 6-hydroxy butanolide autoregulators (see Section 1.3, 1.2-1.10). This empirical correlation between the 2,3-relative configuration and the vicinal coupling constant is based on a  $^1\text{H}$  NMR study by Savostianoff and Pfau<sup>157</sup> in which this relationship was tabulated for 60 variously substituted  $\gamma$ -lactones. They concluded that for 2,3-*cis* configurations the vicinal coupling constants are smaller, ranging from 5-9 Hz, whereas the 2,3-coupling of 2,3-*trans* substituted  $\gamma$ -lactones range from 9-12 Hz. Gräfe and coworkers assumed this relationship to be general and used it to assign the relative 2,3-stereochemistry of isolated 6-hydroxy butanolides (Scheme 4.17). Thus, factor-I (**1.2**) from *S. viridochromogenes*, with a 2,3-coupling constant of 9.4 Hz, was assigned 2,3-*trans*,<sup>26</sup> whereas Gräfe's factors **1.4**, **1.5** and **1.6** from *S. bikiniensis* and *S. cyaneofuscatus* were declared to be 2,3-*cis* due to their smaller 2,3-coupling value (7.3 Hz).<sup>28</sup> Yamada's group has also used this method to assign the 2,3-geometry of the expanding group of butanolide autoregulators isolated from *S. virginiae* (VB's A-E, **1.6-1.10**, see Chapter 1, Section 1.3.3) as well as a range of synthetic analogues.<sup>35</sup>





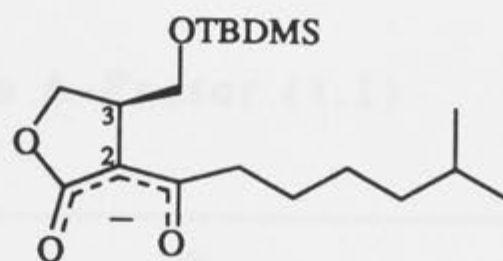
**Scheme 4.17** 2,3-Stereochemistry as Assigned by Gräfe and Coworkers

Although the general applicability and reliability of this method of configurational assignment was questionable, the fact that the 2,3-coupling constant of TBDMS-protected A-factor **4.35** is well within the range reported by Savostianoff and Pfau<sup>157</sup> for 2,3-*cis* configuration made it prudent to re-examine our assumption of 2,3-*trans* geometry. During the catalytic hydrogenation of the butenolide **4.34**, hydrogen is delivered *cis* to the double bond so the 2,3-*cis* lactone **4.35b** is likely to be the initial product. It was conceivable, though improbable, that the *cis*-keto form **4.35b** had actually been isolated rather than being converted into the supposedly lower energy 2,3-*trans* configuration *via* the enol **4.36** (Scheme 4.16). This was considered possible only if:

- the rate of tautomerisation under the reaction and isolation conditions was slow and/or
- the conformational energy of the 2,3-*cis* tautomer **4.35b** is, contrary to expectations, lower than that of the 2,3-*trans* form **4.35a**.

To test these propositions, the silyl lactone **4.35** was fully converted into its enolate anion **4.39** by reaction with an excess of sodium hydride in ether. It was expected that protonation of this would occur substantially at the enolate oxygen to form, initially, the enol **4.36**.<sup>158</sup> Subsequent tautomerisation should give both keto tautomers, provided their conformational energies are similar.





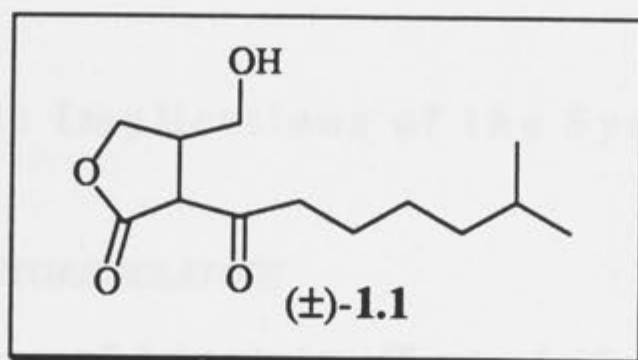
4.39 (enolate)

The product isolated after quenching the enolate with acid was identical by  $^1\text{H}$  NMR to the starting material. It was considered faintly possible that this product was simply the kinetically favoured protonation product and was not necessarily the more thermodynamically stable form, so DBU (*ca* 0.5 eq.) was added to the NMR sample to catalyse the thermodynamic equilibration of the tautomeric components. However, no change was observed by  $^1\text{H}$  NMR spectroscopy after 2 hours at room temperature.

Thus, the isolated keto diastereomer could be the 2,3-*cis* tautomer **4.35b** only if this structure has substantially greater thermodynamic stability than the 2,3-*trans* diastereomer **4.35a**. Although this was considered to be very unlikely given the increased steric interaction expected between the 2,3-*cis* substituents, energy minimisations were conducted for both structures using MM2 force-field calculations as implemented in PC Model (version 88.0). The results from numerous energy minimisations of both structures using a wide variety of initial conformations consistently indicated that the 2,3-*cis* tautomer **4.35b** was between 5 and 10 kcal/mol higher in energy than the 2,3-*trans* form **4.35a**. This result, although not definitive since all possible configurations of all bonds had not been searched, suggests that the observed keto diastereomer of TBDMS-protected A-factor is likely to have 2,3-*trans* relative stereochemistry despite its low 2,3-coupling constant. Thus, the reported correlation between the 2,3-vicinal coupling constant and the relative stereochemistry of 2,3-disubstituted  $\gamma$ -lactones seems highly questionable and the faith shown by other workers in this technique for the assignment of the geometry of the 6-hydroxy butanolide autoregulators and related compounds may have been misplaced. In further studies we have confirmed that this correlation is not reliable, as discussed in Chapter 6.



## 4.3.4 Desilylation to A-Factor (1.1)



Desilylation of TBDMS-protected A-factor **4.35** using glacial acetic acid, THF and water (3 : 3 : 1), as described above in the demethyl reaction series, was again found to proceed slowly. After 48 hours, the appearance of by-products in the reaction mixture (TLC) made it expedient to stop the reaction before completion. On chromatography, 15% of the silyl starting material was recovered followed by A-factor (**1.1**; 71% based on unrecovered starting material) as a colourless oil which formed a waxy solid upon cooling. The yield was later increased dramatically to 95% with the use of the mild and effective reagent system  $\text{HF}_{\text{aq}}$  / pyridine in acetonitrile, as described above for the synthesis of 11-demethyl A-factor **3.36**. The overall yield is 33% over 6 steps from commercially available 1-bromo-4-methylpentane, which compares very favourably with the existing synthetic routes both in yield and simplicity (see Chapter 1, Section 1.6).

Note that racemic synthetic material has *ca* 70% of the activity of pure (3*R*)-A-factor, probably due to partial *in vivo* racemisation occurring *via* translactonisation as mentioned in Section 1.2.<sup>18</sup> Thus in contrast to many natural products, an efficient synthesis of racemic A-factor provides material with activity comparable to that of the enantiomerically pure autoregulator.

Detailed spectroscopic analysis of the product confirmed its identity and also revealed surprising shortcomings in the published data. The interpretation of hitherto unreported features of the spectral data of A-factor has led to the revision of its structure, both in solution and in its pure state, as discussed in the following chapter. That these unreported features are not peculiar to our product was revealed by a spectral



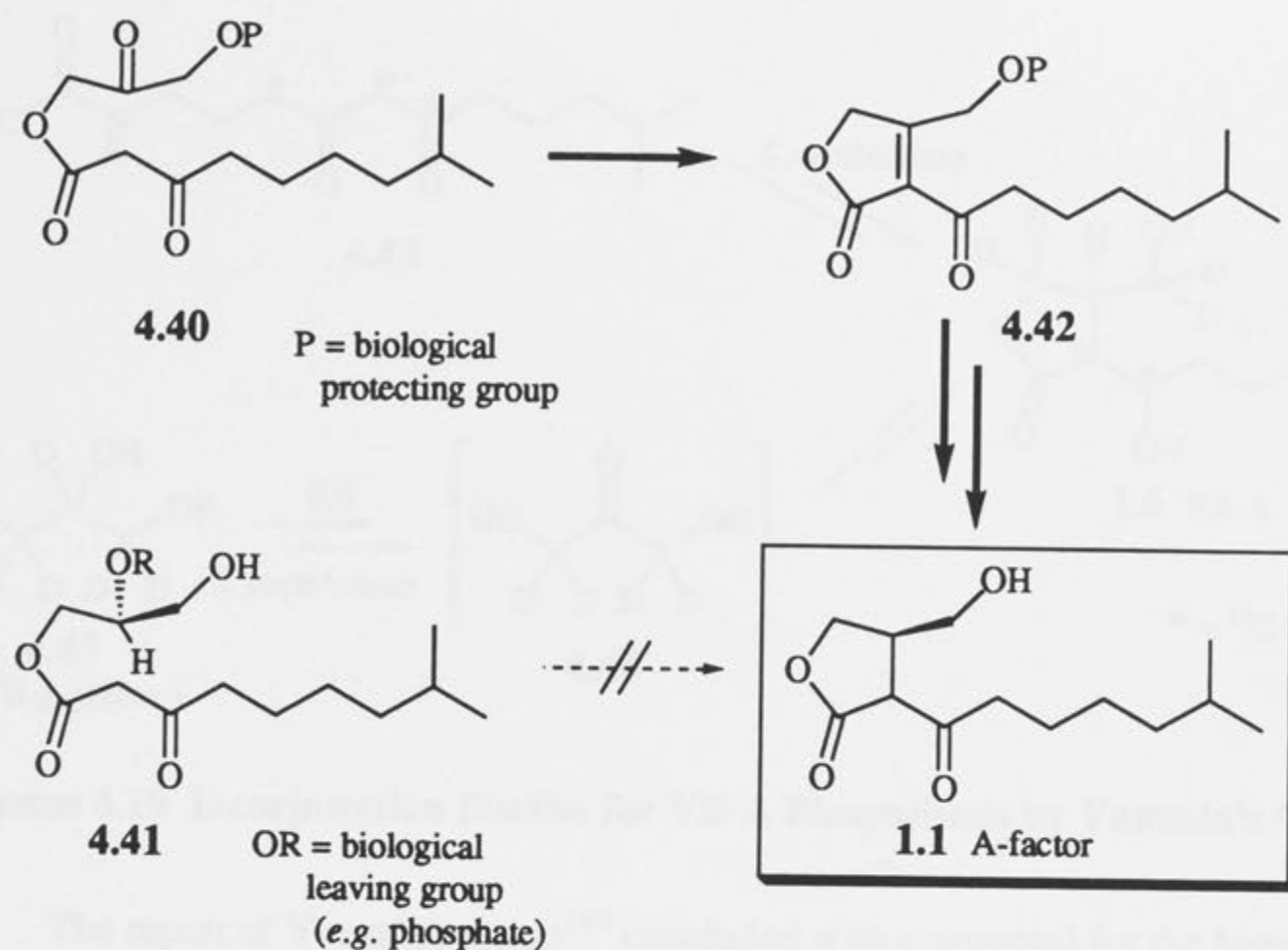
comparison ( $^1\text{H}$  NMR and FTIR) with an authentic sample of A-factor purchased from Funakoshi Co. Ltd.

#### 4.3.5 Biosynthetic Implications of the Synthesis

##### *FOR THE BUTANOLIDE AUTOREGULATORS*

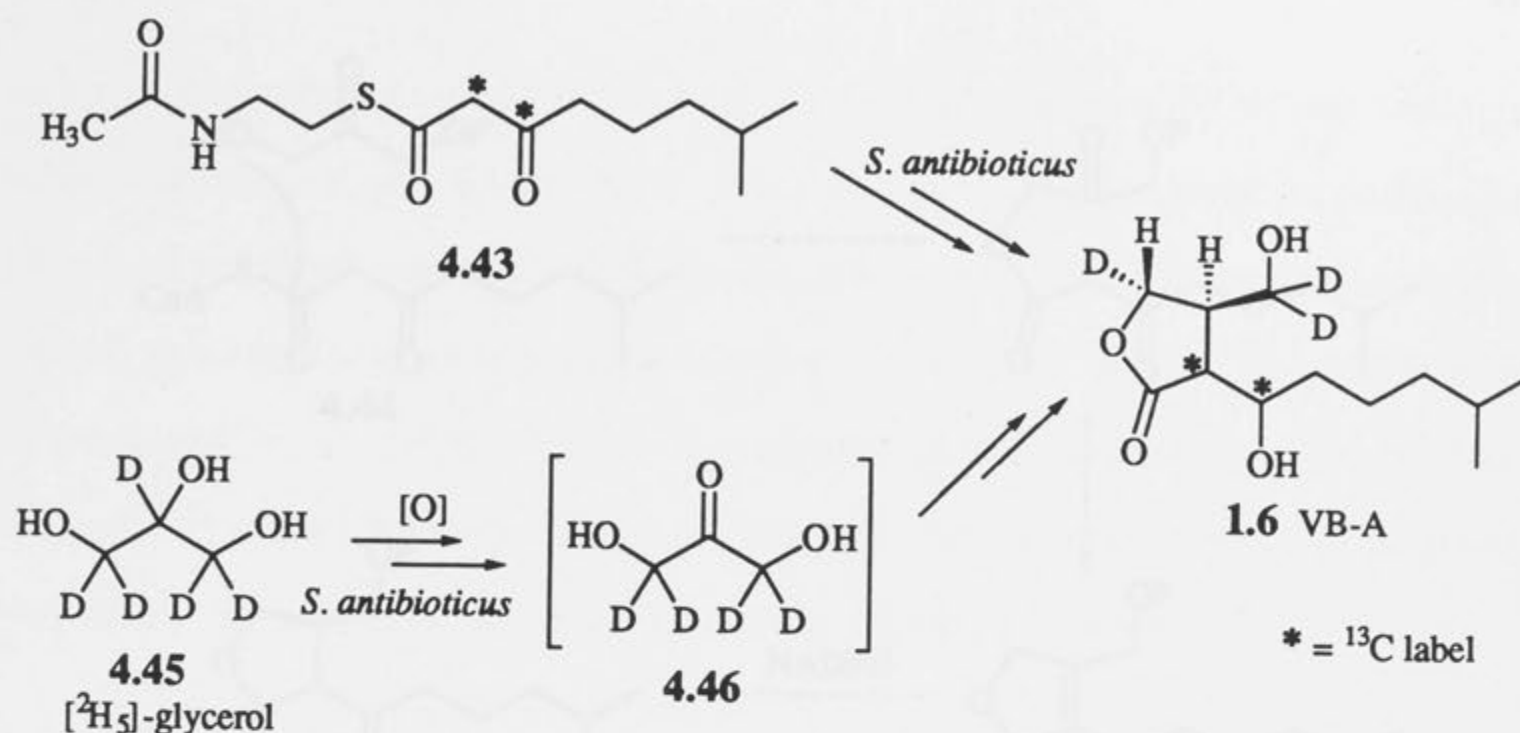
Despite the assurance of Aristotle (see Chapter 4 title page), our discovery of an efficient biomimetic route to A-factor (**1.1**) by no means proves that the biosynthesis of the natural product follows the same path. However, the facility with which we formed the key C2-C3 butanolide bond by Knoevenagel-style cyclisation is in stark contrast with the fundamental stereoelectronic barrier to the desired type of cyclisation which we identified during investigation of the other potentially biomimetic route involving intramolecular alkylation (Chapter 3). Hence, assuming that the C2-C3 bond of A-factor is biosynthesised *via* an intramolecular process, it appears far more likely that it would be achieved through the cyclisation of the  $\beta$ -ketoester of a dihydroxyacetone derivative **4.40** rather than *via* the corresponding glycerol-type substrate **4.41** (Scheme 4.18). It is possible that the free primary hydroxyl group of the cyclisation precursor **4.40** would have some form of biological protection (P) in view of the instability we observed for the unprotected butenolide intermediate. The chirality of A-factor is thus likely to derive from the asymmetric reduction of the intermediate butenolide **4.42** (probably with NADPH) rather than from the pre-established chirality of a glycerol-type precursor **4.41**. Confirmation of the intermediacy of the butenolide **4.42** might be gained by feeding studies using labelled versions of the intermediate **4.42** with A-factor producing organisms. An intriguing possibility for future study would be the use of non-producing organisms such as bakers yeast<sup>159</sup> to effect the *in vivo* asymmetric reduction of synthetically produced butenolide **4.42** to optically active A-factor (**1.1**).





Scheme 4.18

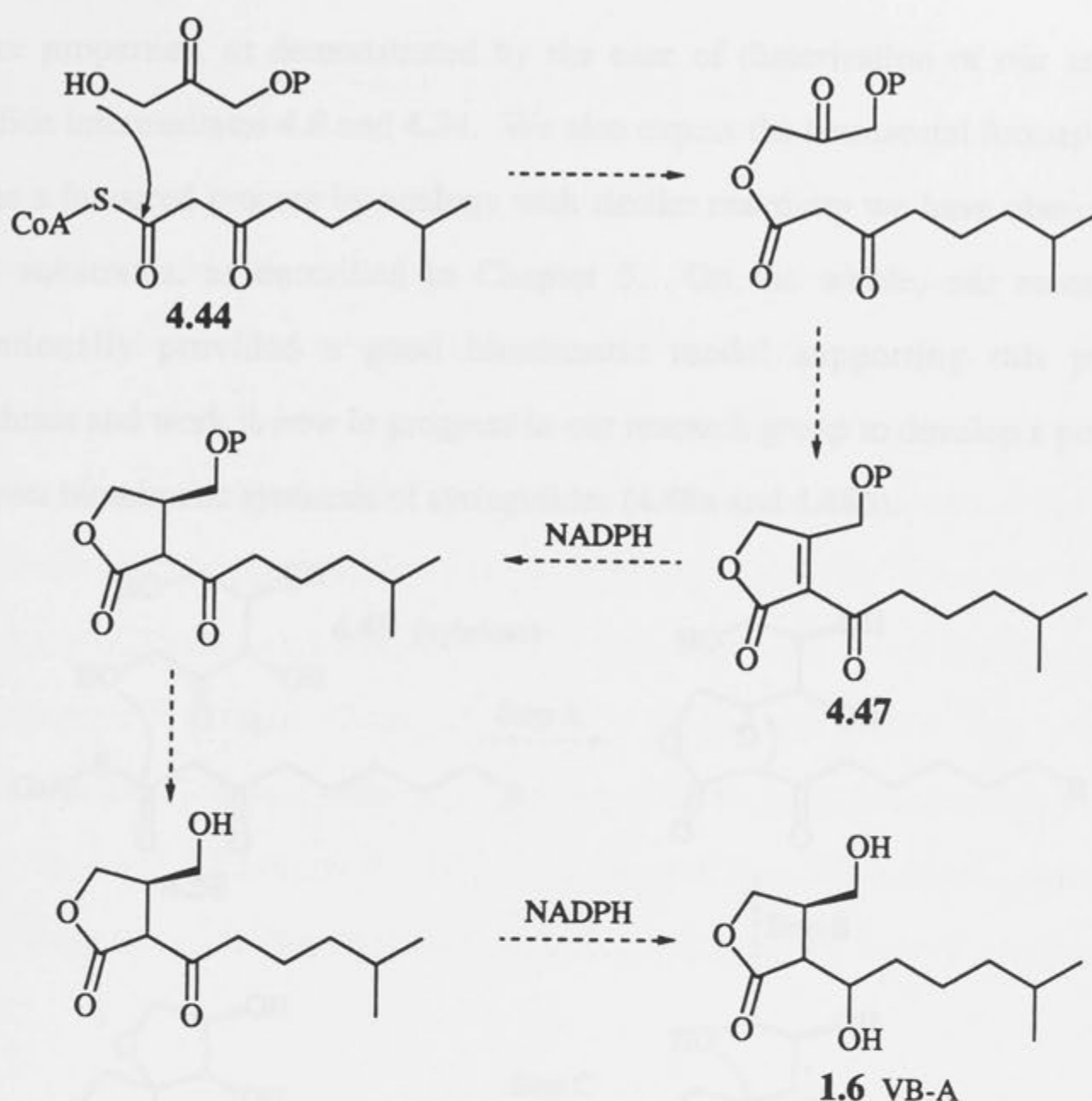
Very recently, Yamada and coworkers published further evidence for the biosynthesis of VB-A (**1.6**),<sup>160</sup> extending their earlier feeding studies<sup>87</sup> which established the biological origins of its carbon skeleton, (see Chapter 2, Section 2.3). The new work confirmed the biological intermediacy of a  $\beta$ -ketoacid derivative by the intact incorporation into VB-A (**1.6**) of the doubly  $^{13}\text{C}$ -labelled  $\beta$ -ketothioester **4.43**, (Scheme 4.19) a mimic of the expected enzyme bound biosynthetic intermediate **4.44** (Scheme 4.20). In a further experiment, they found that incorporation of  $[^2\text{H}_5]$ -glycerol (**4.45**) resulted in the formation of VB-A (**1.6**) which lacked deuterium at C3, suggesting that the glycerol moiety had been oxidised to a dihydroxyacetone derivative e.g. **4.46** prior to the formation of the butanolide C2-C3 bond (Scheme 4.19). The loss of another deuterium label at C4 (*pro-S*) was rationalised by an enzyme catalysed exchange process of the putative butenolide intermediate **4.47** (Scheme 4.20).



**Scheme 4.19** Incorporation Studies for VB-A Biosynthesis by Yamada's Group

The report of Yamada's group<sup>160</sup> concluded with a proposal for the biosynthesis of VB-A (**1.6**) (Scheme 4.20) which is directly analogous to our biomimetic route. A preliminary report of our biomimetic synthesis of 11-demethyl A-factor (**3.36**)<sup>131</sup> was cited as evidence for the feasibility of the key cyclisation step.



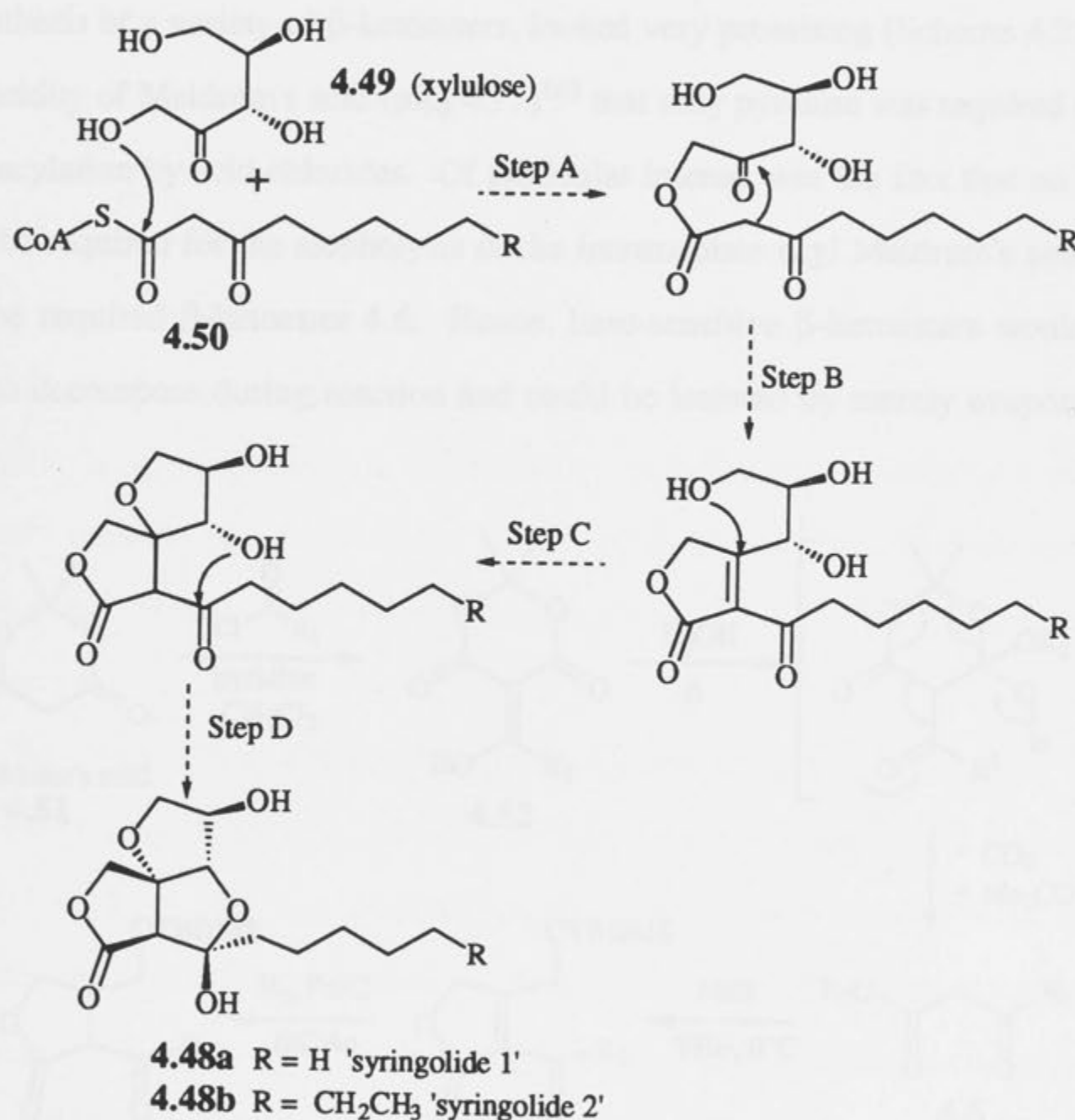


**Scheme 4.20** Proposed Biosynthesis of VB-A (1.6) by Yamada's Group

#### BIOSYNTHETIC IMPLICATIONS FOR THE SYRINGOLIDES

Very recently, Midland and coworkers reported the isolation of two novel bacterial signal molecules, 'syringolides 1 and 2' (4.48a and 4.48b), from *Pseudomonas syringae* pv. *tomato*. These have been demonstrated to elicit a defence response in infected soybean plants.<sup>161</sup> The report includes a proposed biosynthetic route to these compounds (Scheme 4.21), starting with the acylation of D-xylulose (4.49) with a  $\beta$ -ketoacid derivative 4.50 (Step A, Scheme 4.21). The Knoevenagel-style cyclisation (Step B) is analogous to the key cyclisation of our biomimetic route to A-factor and was in fact modelled on the above biosynthetic proposal for VB-A (1.6), reported by Yamada's group<sup>160</sup> (Scheme 4.20). We feel that the subsequent Michael addition to the butenolide moiety (Step C) is likely to be facile in view of the strong Michael

acceptor properties, as demonstrated by the ease of dimerisation of our analogous butenolide intermediates **4.8** and **4.34**. We also expect the hemiacetal formation (Step D) to be a favoured process by analogy with similar reactivity we have observed with related substrates, as described in Chapter 5. On the whole, our research has unintentionally provided a good biomimetic model supporting this proposed biosynthesis and work is now in progress in our research group to develop a potentially very direct biomimetic synthesis of syringolides (**4.48a** and **4.48b**).

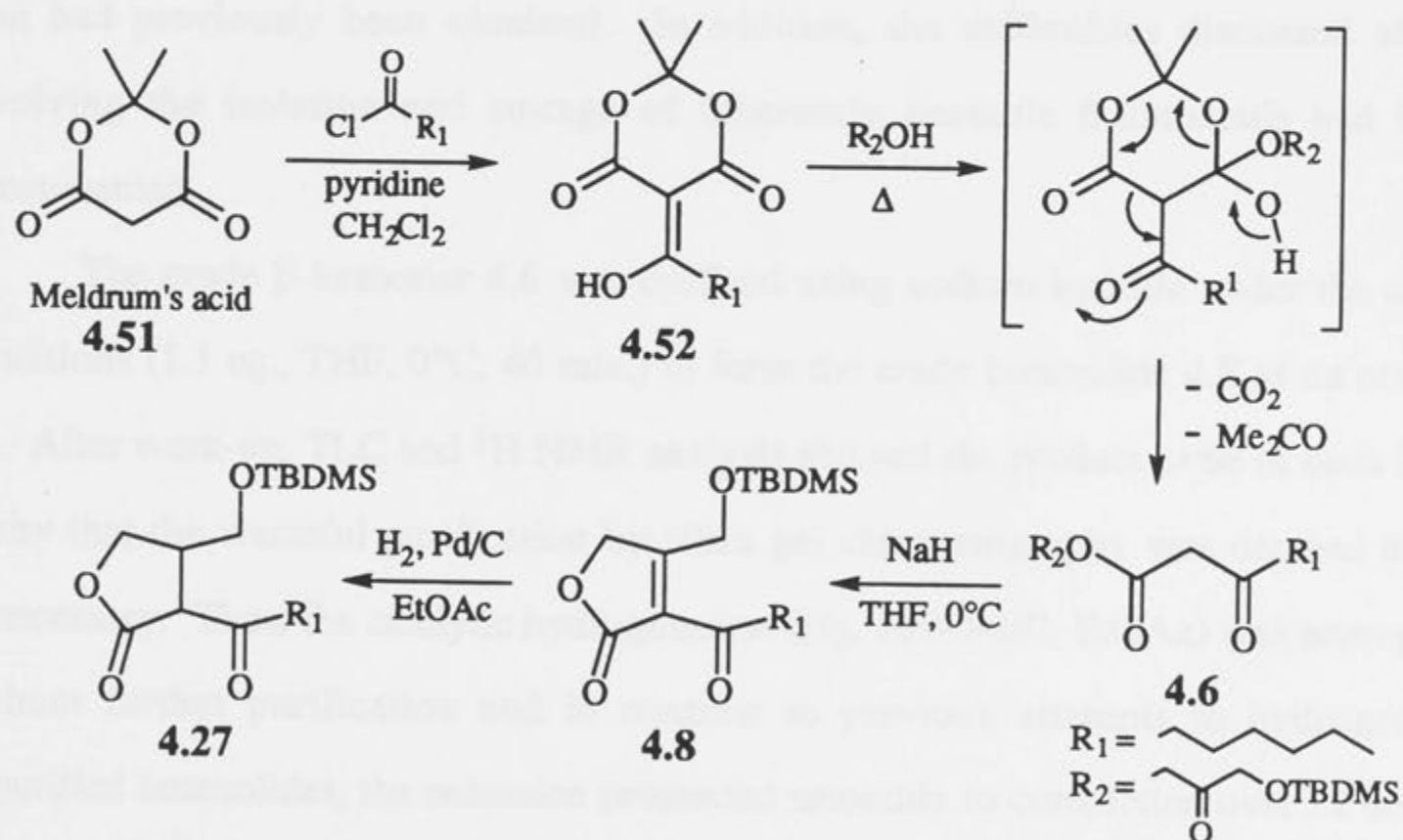


**Scheme 4.21** Proposed Biosynthesis of Syringolides 1 and 2 by Midland *et al.*



#### 4.4 Postscript: A Synthetic Short Cut

Although a simple and efficient synthetic route to A-factor (**1.1**) and its homologue **3.36** had been discovered, it was felt that it could be further improved to make the synthesis more convenient, especially on a large scale. Thus an alternative route to the key  $\beta$ -ketoester intermediates **4.6** or **4.33** was sought and the elegant method developed by Oikawa and coworkers,<sup>162</sup> which uses Meldrum's acid (**4.51**) for the synthesis of a variety of  $\beta$ -ketoesters, looked very promising (Scheme 4.22). Such is the acidity of Meldrum's acid ( $pK_a$  4.97)<sup>163</sup> that only pyridine was required as a base for its acylation by acid chlorides. Of particular interest was the fact that no catalysis would be required for the alcoholysis of the intermediate acyl Meldrum's acid **4.52** to form the required  $\beta$ -ketoester **4.6**. Hence, base-sensitive  $\beta$ -ketoesters would be less likely to decompose during reaction and could be isolated by merely evaporating the solvent.



Scheme 4.22

During the preparation of this report, it was decided to quickly assess the viability of this attractive, modified approach by its application to the synthesis of 11-demethyl A-factor **4.27** as outlined in Scheme 4.22.



The acylation of Meldrum's acid with heptanoyl chloride was complete within 1 hour at room temperature, giving the crude heptanoyl Meldrum's acid **4.52** as an orange oil.  $^1\text{H}$  NMR showed this product to be of high purity and confirmed that it exists entirely in the enol form. The enol hydroxyl appeared as a sharp, strongly deshielded singlet at  $\delta$  15.2. The crude intermediate **4.52** was subjected to alcoholysis with 2.5 equivalents of TBDMS-protected dihydroxyacetone **4.7** in benzene solution. After 2 hours at reflux, no intermediate **4.52** was detected by TLC so the solvent was evaporated. The unreacted silyl dihydroxyacetone **4.7** was removed from the product mixture by Kugelrohr distillation (oven  $100^\circ\text{C}$ , 0.1 mm Hg). The residual yellow-orange oil contained the  $\beta$ -ketoester **4.6** in an overall yield of *ca* 65% from Meldrum's acid, as estimated by  $^1\text{H}$  NMR integration using an internal integration standard (diphenylmethanol). This is a substantial improvement over the best  $\beta$ -ketoester yield attained *via* our previous 3-step methods (*cf.*  $\beta$ -ketoester **4.33**, *ca* 48% overall from *t*-butyl acetoacetate) and the crude product is of considerably higher purity than had previously been obtained. In addition, the difficulties discussed above involving the isolation and storage of inherently unstable  $\beta$ -ketoacids had been circumvented.

The crude  $\beta$ -ketoester **4.6** was cyclised using sodium hydride under the usual conditions (1.1 eq., THF,  $0^\circ\text{C}$ , 40 min.) to form the crude butenolide **4.8** as an orange oil. After work-up, TLC and  $^1\text{H}$  NMR analysis showed the product to be of such high purity that the wasteful purification by silica gel chromatography was deemed to be unnecessary. Thus, the catalytic hydrogenation ( $\text{H}_2$ , 10% Pd/C, EtOAc) was attempted without further purification and in contrast to previous attempts to hydrogenate unpurified butenolides, the reduction proceeded smoothly to completion over 12 hours. TBDMS-protected 11-demethyl A-factor **4.27** was isolated after radial chromatography in an overall yield from Meldrum's acid **4.51** of 48%. Since the subsequent desilylation to demethyl A-factor **3.36** has been previously performed in 90% yield ( $\text{HF}_{\text{aq}}$  / pyridine /  $\text{CH}_3\text{CN}$ ), the expected overall yield of demethyl A-factor **3.36**, after a single trial of this route, is *ca* 43%. Although time did not permit the optimisation of this



dramatically improved variation of our synthetic route or its application to the synthesis of A-factor itself (1.1), there is no reason to suspect that the route is not of general applicability and that similar or improved yields of A-factor itself (1.1) and its other homologues could be attained. This route represents a substantial improvement on all existing syntheses of this group of compounds (*cf.* Chapter 1, Section 1.6), due to:

- the higher overall yield;
- its simplicity, only five steps being required with only one intermediate needing chromatographic purification. All reagents are readily available and easily handled;
- its convenience; all reactions are performed at room temperature or 0°C and the full reaction series requires less than 2 days, even on a large scale.

With (±) A-factor recently becoming a commercial product commanding a price of *ca* A\$100 *per milligram*,<sup>22</sup> these factors make our process an excellent candidate for development as a commercial process.

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### CHAPTER FIVE

#### A-FACTOR REVISITED

*Nature is not embarrassed by difficulties in analysis*

Augustine Jean Fresnel (1785-1827)



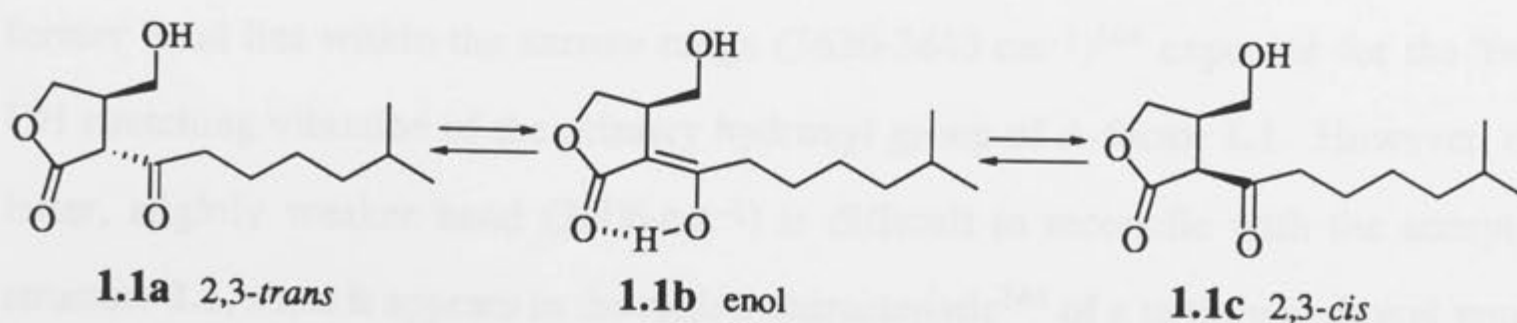
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## 5.1 INTRODUCTION

Khokhlov and coworkers established that A-factor was a 2,3-disubstituted  $\gamma$ -lactone which they expected to be readily epimerisable at the C2 position *via* the enol form **1.1b**.<sup>9</sup> They assumed that the autoregulator existed exclusively in the 2,3-*trans* form **1.1a**, since this was presumed to be thermodynamically more stable than the 2,3-*cis* isomer **1.1c**. Their assignment of the absolute configuration at C2 followed from this assumption of *trans* stereochemistry and their (erroneous) determination of the absolute configuration of C3 (see Section 1.2). A minor but unquantified proportion of the compound was shown to exist in the enol form **1.1b** in solution by UV spectroscopy. No other structural forms of A-factor were discussed. Mori and coworkers agreed with Khokhlov that the 2,3-*trans* isomer **1.1a** "seems to be more thermodynamically stable" than the 2,3-*cis* form **1.1c**, but no supporting evidence was presented.<sup>18</sup> Mori and coworkers were, however, more cautious than Khokhlov *et al.*, declaring that "the configuration at that position (C2) is uncertain". Possible contributions of other isomeric forms were not discussed. Interestingly Gräfe *et al.* reported that a sample of synthetic ( $\pm$ )-A-factor **1.1**, obtained from Khokhlov for bioassay, was "according to its 200 MHz  $^1\text{H}$  NMR spectrum a mixture of 2,3-*cis* and *trans* isomers".<sup>26</sup> Although neither the  $^1\text{H}$  NMR data nor the isomeric ratio was reported, this comment implies a significant contribution ( $> ca\ 5\%$ ) by the 2,3-*cis* form **1.1c** which was not noted by Khokhlov or Mori. Subsequent reports of the synthesis of A-factor and homologues have failed to contribute any new evidence relating to the identity or the relative contributions of isomeric structures, despite the use of high resolution  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy.<sup>20,29,35</sup>





In this chapter, a re-examination of the spectroscopic properties of A-factor reveals its isomeric composition to be considerably more complex than has hitherto been assumed.

## 5.2 SPECTROSCOPIC ANALYSIS OF A-FACTOR

The spectral data supporting the previously reported syntheses of A-factor<sup>18,20,29,51</sup> **1.1** vary considerably, both in the quality of data and in the accuracy of interpretation. Where reported the <sup>1</sup>H NMR data, in particular, are of limited value due to the low resolution and/or the inaccuracy of assignments.<sup>18,20</sup> We found these shortcomings surprising for such an extensively studied molecule, so a comprehensive review of the IR, UV, MS and NMR spectral data of A-factor **1.1** was undertaken.

### 5.2.1 Infrared Analysis

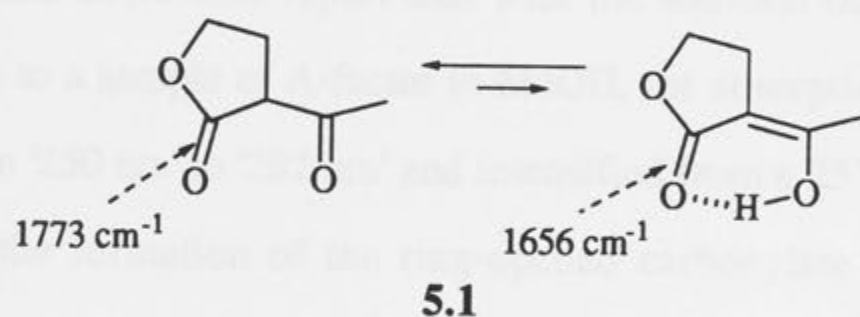
All reported infrared data for A-factor **1.1** have been for thin films, a technique which tends to compromise resolution and give spectra which are influenced by intermolecular hydrogen bonding.<sup>18,29,51</sup> Under these conditions, hydrogen bonding reduces the  $\gamma$ -lactone carbonyl stretching frequency from 1782 cm<sup>-1</sup>, the diagnostic position which we observe in CCl<sub>4</sub> solution, to 1765<sup>18,29</sup> or 1770<sup>15</sup> cm<sup>-1</sup>. It may have been this shift to a less definitive frequency which delayed the identification of natural A-factor **1.1** as a  $\gamma$ -lactone during the initial investigation of its primary characteristics,<sup>15</sup> until degradation studies were performed.<sup>9</sup>

As a thin film, intermolecular hydrogen bonding gives rise to a broad OH stretching absorption centred at 3460 cm<sup>-1</sup>, whereas in CCl<sub>4</sub> solution we observed two sharp absorbances at 3639 and 3606 cm<sup>-1</sup> in addition to this broad absorption. The former band lies within the narrow range (3630-3643 cm<sup>-1</sup>)<sup>144</sup> expected for the 'free' OH stretching vibration of the primary hydroxyl group of A-factor **1.1**. However, the latter, slightly weaker band (3606 cm<sup>-1</sup>) is difficult to reconcile with the accepted structure **1.1**, since it appears in the region characteristic<sup>144</sup> of a tertiary hydroxyl group



(3600-3620  $\text{cm}^{-1}$ ). The significance of this band was revealed by further chemical and spectroscopic studies described below (Section 5.3.3), which resulted in the reassessment of the structure of A-factor in solution.

Major infrared absorbances of A-factor in the 850-1750  $\text{cm}^{-1}$  region, recorded as a thin film,<sup>18,29</sup> are in good agreement with those we observed for our product in  $\text{CCl}_4$  solution ( $\pm 5 \text{ cm}^{-1}$ ). The ketone carbonyl band appears at 1720  $\text{cm}^{-1}$  and a pair of absorptions at 1367 and 1383  $\text{cm}^{-1}$  are presumably the characteristic  $\text{CH}_3$  bending absorptions of the *gem*-dimethyl group.<sup>144</sup> In the otherwise very similar spectrum of 11-demethyl A-factor **3.36**, this doublet was replaced by a single absorbance at 1380  $\text{cm}^{-1}$ . It is likely that the weak band at 1647  $\text{cm}^{-1}$ , also reported by Kinoshita and Hirano,<sup>29</sup> is due to the chelated lactone carbonyl stretching absorption of the enol form of A-factor **1.1b**. Similar bands, attributed to this vibration, have been reported for  $\alpha$ -acetyl- $\gamma$ -butyrolactone **5.1** (see below) and for several  $\beta$ -ketoesters.<sup>164</sup> Other peaks in the 'fingerprint' region were not unambiguously assigned but their similarity with the reported data served to further confirm the identity of our product.



### Literature Model for Lactone Carbonyl Stretching Frequency

A sample of racemic A-factor which was subsequently obtained from Funakoshi Co. Ltd. exhibited an IR spectrum in  $\text{CCl}_4$  solution identical to that obtained for our product.

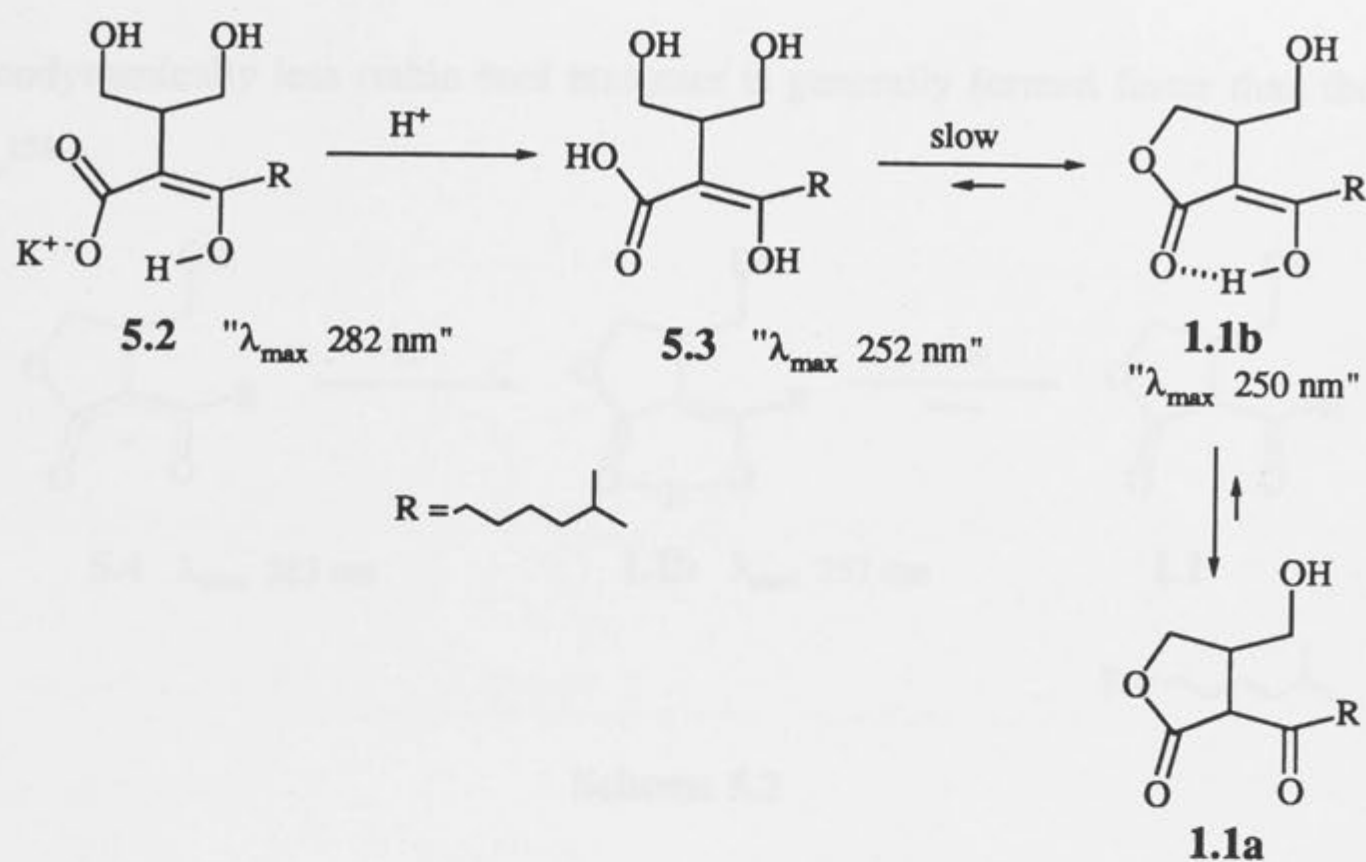


### 5.2.2 Ultraviolet Analysis

In neutral or mildly acidic MeOH solution, A-factor exhibits a single maximum at 257 nm ( $\epsilon$  1200) which we attribute to the  $\pi$ - $\pi^*$  transition of the enolic form **1.1b**. This band completely obscures the very weak n- $\pi^*$  absorptions ( $\epsilon < 30$ ) which may have been expected for the carbonyl groups of the keto form **1.1a**. The absorption bands at 206 nm<sup>15</sup> and 226 nm<sup>9</sup> reported by Khokhlov's group for the naturally derived material in MeOH, are evidently due to the presence of impurities. We found that the presence of adventitious traces of base in the UV solution caused a second band of variable intensity to appear at 283 nm. This band disappeared with the addition of a trace of acid and was presumed to be due to the partial formation of the enolate anion **5.4**, since  $\beta$ -dicarbonyl compounds in general are known to form enolate ions which absorb in this region.<sup>103,165,166</sup> This is likely to be the origin of a second, unassigned maximum at 283.5 nm ( $\epsilon$  250) which was reported by Mori (solvent not specified), in addition to the dominant band [255 nm ( $\epsilon$  855)].<sup>17</sup>

Khokhlov and coworkers report that with the addition of aqueous potassium hydroxide solution to a sample of A-factor in MeOH, the absorption maximum shifted in wavelength from '250 nm' to '282 nm' and intensified from  $\epsilon$  2330 to  $\epsilon$  13000. They attributed this to the formation of the ring-opened carboxylate anion **5.2**.<sup>9</sup> Upon acidification, a hypsochromic shift back to '252 nm' was observed, initially forming an intense band ( $\epsilon$  5500), which then steadily diminished to the intensity of the original enol absorbance ( $\epsilon$  1800) after 30 minutes. They suggested that the initially large peak at '250 nm' was due to the formation of the carboxylic acid **5.3** which then slowly lactonises to reform the original keto-enol mixture **1.1a-1.1b**, with an attendant drop in intensity (Scheme 5.1). No direct evidence for the ring-opening was provided but, in a separate experiment, A-factor was treated for an unspecified period with ethanolic KOH, (EtOH : 0.01 M KOH<sub>aq</sub>, 1:1, 20°C), to form a product which was assigned, by IR spectroscopy alone, as the potassium salt of the ring-opened carboxylate **5.2**. No UV data was reported for the product and its relactonisation to A-factor was not described.



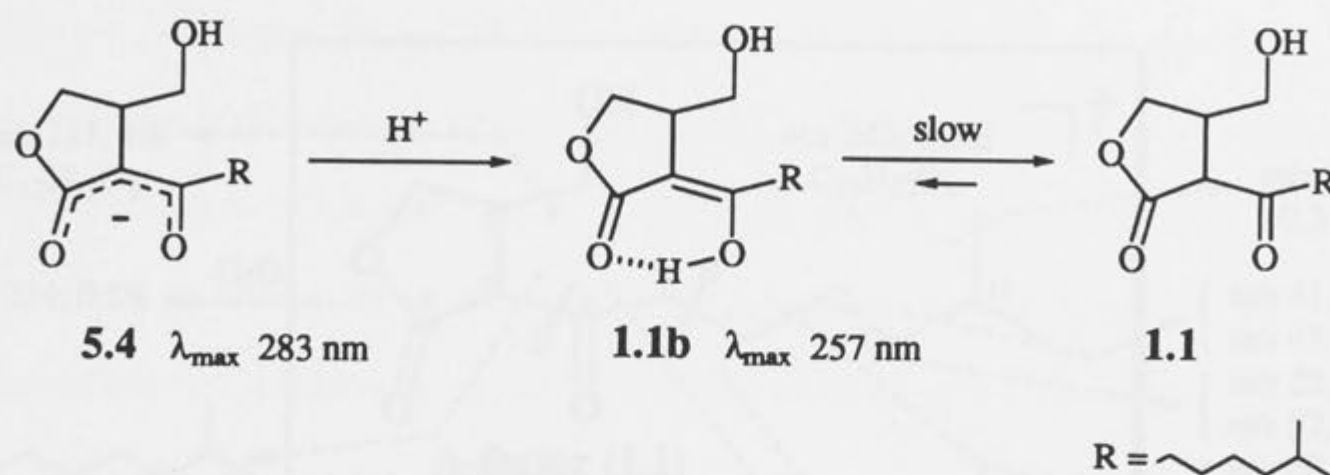


**Scheme 5.1 Rationalisation of UV Data According to Khokhlov**

We also observed that the acidification of a basic solution of A-factor (0.005 M NaOH / MeOH), caused a hypsochromic shift from 283 nm ( $\epsilon$  20100) to 257 nm ( $\epsilon$  7700) and that the resulting peak gradually attenuated, over 15 minutes, to an intensity compatible with that of the enol content of A-factor in MeOH ( $\epsilon$  1200). We also noted analogous behaviour for the TBDMS protected precursor to A-factor **4.27** and contend that this behaviour can be explained without invoking the opening and reclosure of the lactone ring. While it is likely that  $\gamma$ -lactone opening will eventually occur in the presence of hydroxide, it is considered improbable that this would happen almost instantaneously at room temperature, as would be required to explain the observations during UV spectroscopy. It appears more likely that the large peak at 283 nm, observed in alkaline solution, is simply due to formation of the enolate anion **5.4** and that upon acidification, protonation of the enolate occurs preferentially on the C6 oxygen (rather than at C3) to initially form substantially more of the enol form **1.1b** than normally exists in an equilibrated solution of A-factor in methanol. Subsequently, the absorbance at 257 nm due to the enhanced levels of enol form **1.1b**, diminishes as the normal keto-enol equilibrium is re-established (Scheme 5.2). In support of this alternative explanation is the observation by Eigen, in his report on proton transfer processes, that during protonation of the enolate anions of  $\beta$ -dicarbonyl compounds, the



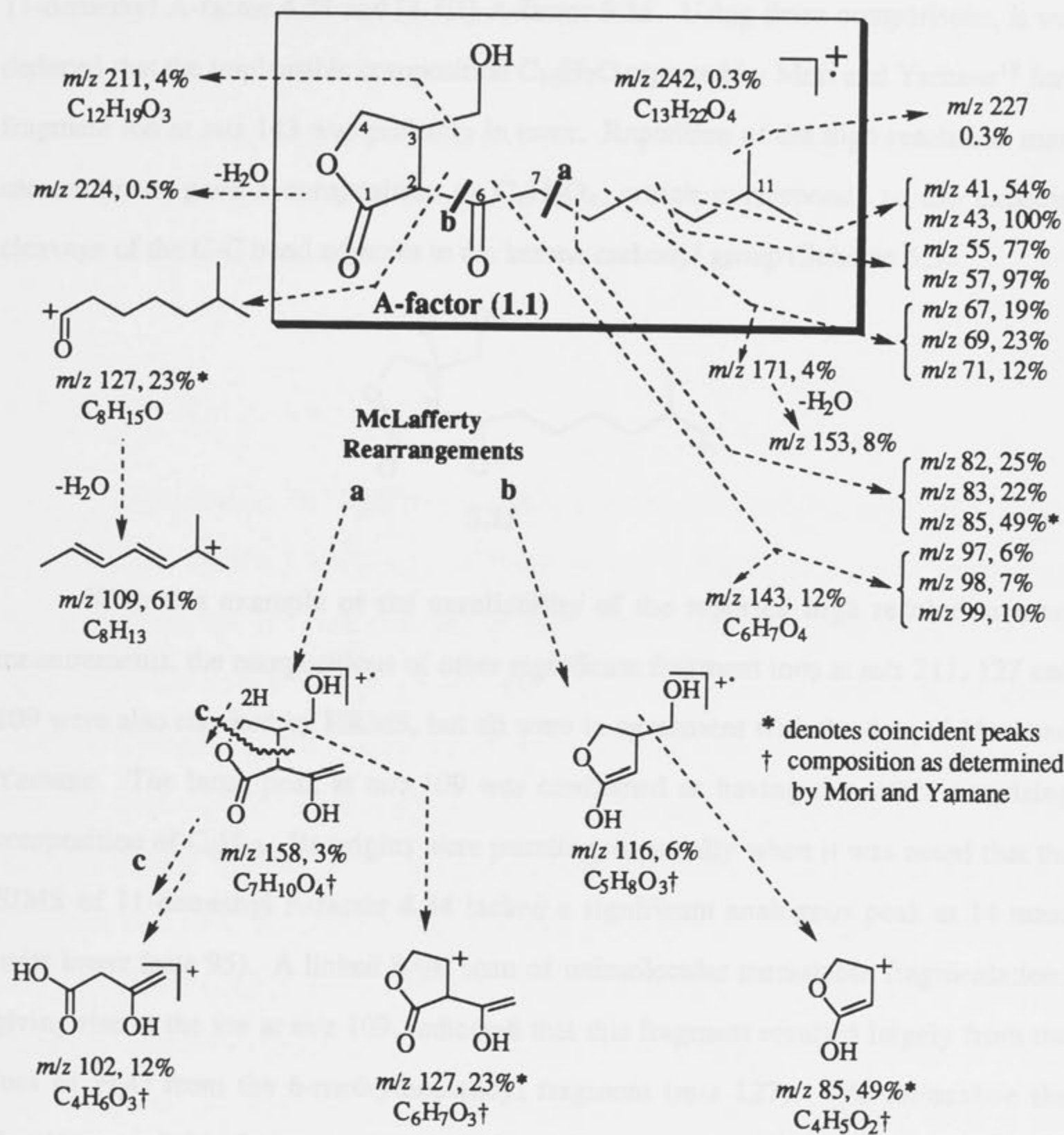
thermodynamically less stable enol tautomer is generally formed faster than the keto form.<sup>158</sup>



Scheme 5.2

### 5.2.3 Mass Spectral Analysis

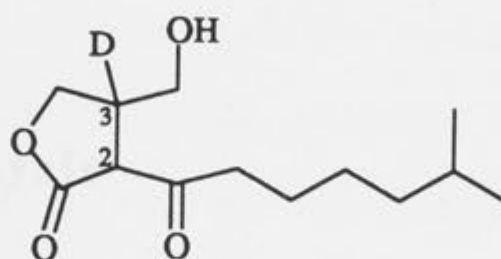
The EI mass spectrum of natural (-)-A-factor was reported by Khokhlov's group<sup>15</sup> and is, apart from some minor peaks presumably arising from impurities in the natural material, very similar to the spectrum obtained for our synthetic product. Their spectrum was originally used to establish the molecular weight of A-factor ( $m/z$  242,  $\text{M}^+$ ) and to confirm the existence of a hydroxymethyl substituent ( $m/z$  211,  $\text{M}^+ - \text{CH}_2\text{OH}$ ), but no other peak assignments were made at the time. Mori and Yamane<sup>18</sup> have subsequently determined the elemental composition of twenty one of the most significant peaks in the mass spectrum of their synthetic (-)-A-factor by high resolution mass measurements, but did not attempt to rationalise the formation of these fragments. One major fragment at  $m/z$  141 (28%) appears to be an artefact since it was reported to have the unlikely composition of  $\text{C}_{11}\text{H}_9$ , and is not observed in our EIMS spectra or in that of Khokhlov and coworkers. Since some of the other reported peak compositions were also difficult to explain and other peak intensities differed significantly from those exhibited by our product, we sought to rationalise the mass spectral fragmentations of A-factor, as depicted in Scheme 5.3.



Scheme 5.3 Mass Spectral Fragmentation of A-factor



The depicted homolytic cleavages are supported by the appearance of appropriate homologous peaks in the mass spectra of the closely related compounds 11-demethyl A-factor **4.34** and [3-<sup>2</sup>H]-A-factor **5.15**. Using these comparisons, it was deduced that the implausible composition C<sub>10</sub>H<sub>7</sub>O reported by Mori and Yamane<sup>18</sup> for a fragment ion at *m/z* 143 was probably in error. Repetition of the high resolution mass measurement gave a composition as C<sub>6</sub>H<sub>7</sub>O<sub>4</sub>, which corresponds to the expected cleavage of the C-C bond adjacent to the ketone carbonyl group (Scheme 5.3).

**5.15**

With this example of the unreliability of the reported high resolution mass measurements, the compositions of other significant fragment ions at *m/z* 211, 127 and 109 were also checked by HRMS, but all were in agreement with the data of Mori and Yamane. The large peak at *m/z* 109 was confirmed as having the rather surprising composition of C<sub>8</sub>H<sub>13</sub>. Its origins were puzzling, especially when it was noted that the EIMS of 11-demethyl A-factor **4.34** lacked a significant analogous peak at 14 mass units lower (*m/e* 95). A linked B<sup>2</sup>/E scan of unimolecular metastable fragmentations giving rise to the ion at *m/z* 109, indicated that this fragment resulted largely from the loss of H<sub>2</sub>O from the 6-methylheptanoyl fragment (*m/z* 127). We rationalise the dominance of this fragmentation path for A-factor, by the formation of the highly stabilised tertiary allylic carbonium ion, as depicted in Scheme 5.3.

McLafferty rearrangements (**a** and **b**, Scheme 5.3) generate the hydroxymethylbutyrolactone fragments at *m/z* 116 and *m/z* 158, both of which may then lose a CH<sub>2</sub>OH fragment to give major peaks at *m/z* 85 and at *m/z* 127, respectively.

The fragment at *m/z* 102 (C<sub>4</sub>H<sub>6</sub>O<sub>3</sub>) also appears in the spectrum of [3-<sup>2</sup>H]-A-factor, **5.15** suggesting that carbon 3 is probably not present in the fragment. Since three oxygen atoms are present, it appears likely that the fragment represents carbons



1,2,6 and 7. A fragment with the required composition could be formed from the radical cation  $m/z$  158 via a process involving the cleavage of two bonds (C2-C3 and C4-O) with the associated transfer of two hydrogen atoms, as depicted in Scheme 5.3, (cleavage c).

Note that the existence of tautomeric forms of A-factor (described in detail later in this chapter) means that some fragments may have different (or mixed) origins from those depicted in Scheme 5.3.

#### 5.2.4 $^1\text{H}$ NMR Analysis

The published  $^1\text{H}$  NMR data for A-factor are of remarkably poor quality. The 60 MHz data reported by Mori and Yamane<sup>18</sup> in 1982 without an accompanying spectrum, was not assigned and was of such poor resolution as to make it of very limited use for comparison purposes. The data were presented largely as integrated 'multiplet' ranges with no coupling information, and even this limited information contains three significant errors (Figure 5.1). The multiplet regions extend only to  $\delta$  4.2 and thus exclude the H4a proton of the keto form of A-factor **1.1a** which we have found to appear at  $\delta$  4.43. In addition, the region  $\delta$  3.5-3.8 is reported to contain two protons when it in fact contains three (**1.1a**, H2, H5a and H5b), and the region  $\delta$  1.0-2.0 which is said to represent 9 protons, actually contains 7 (or possibly 8 if the hydroxyl proton was present in this region). Reports of subsequent syntheses of A-factor, up to as late as 1992, have uncritically accepted this data and declared their product to exhibit identical NMR spectra.<sup>20,29,30</sup> Posner and coworkers,<sup>20</sup> in their report of the asymmetric synthesis of (+)-A-factor, included their own 400 MHz  $^1\text{H}$  NMR data (Figure 5.1), again with no spectrum but with still more errors, apparently resulting from an attempt to reconcile their spectrum with the flawed data of Mori. Despite the high field strength used, their spectral information is also presented as unassigned 'multiplet' regions with no coupling data, and again no peaks downfield of  $\delta$  4.2 are reported. In common with Mori's data, the region ' $\delta$  3.800-3.661' is incorrectly reported



to represent only two protons where three actually appear. In addition, the region ' $\delta$  3.103-2.61' is stated to encompass three protons when in fact only two appear (1.1a, H7a and H7b). Given these significant departures from our spectral data, it was surprising to find that when a copy of the actual spectrum was later obtained from Posner, it was virtually identical to ours (Figure 5.1) apart from the presence of some solvent peaks. A sample of ( $\pm$ )-A-factor subsequently obtained from Funakoshi Co. Ltd., presumably prepared by a method related to that of Mori and Yamane,<sup>18</sup> also displayed a  $^1\text{H}$  NMR spectrum essentially identical to ours.

Clearly, the literature provided little assistance in the assignment of high field  $^1\text{H}$  NMR spectra of A-factor or even for the confirmation of the structure of our product. Fortunately, we had previously comprehensively studied TBDMS protected A-factor 4.35 by NMR, as described in the preceding chapter, and this provided an excellent NMR comparison. A-factor in  $\text{CDCl}_3$  displayed, *inter alia*, a set of peaks which corresponded closely to the spectrum of TBDMS protected 2,3-*trans*-A-factor 4.35, and accordingly these resonances have been assigned to the 2,3-*trans* keto form of A-factor 1.1a, as shown in yellow in Figure 5.1. Disturbingly, the spectrum also displayed a complex array of unidentified secondary peaks displayed in blue in Figure 5.1, representing approximately 40% of the sample. The expected enol form 1.1b accounted for only about 4% of the sample, as indicated by the integration of the distinctive enol OH resonance at  $\delta$  11.2 (not shown in Figure 5.1). The complexity of these secondary peaks suggested the presence of at least two other chemical species. These 'extra' peaks persisted even in samples of A-factor which had undergone further, careful purification by silica gel chromatography and reverse phase MPLC (RP8, 82.5% MeOH in  $\text{H}_2\text{O}$ ).

#### 5.2.5 $^{13}\text{C}$ NMR Analysis

$^{13}\text{C}$  NMR analysis revealed the presence of a mixture of compounds even more clearly. The major set of peaks corresponded very closely (within 0.5 ppm) to the  $^{13}\text{C}$  NMR data for A-factor reported by Mori and Yamane<sup>18</sup> and Kinoshita and Hirano.<sup>29</sup>

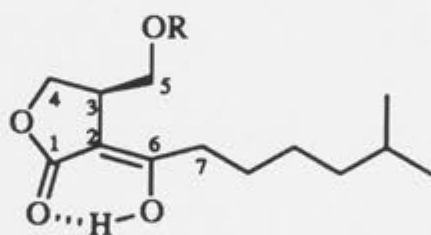


Figure 5.1 Comparison of <sup>1</sup>H NMR Spectrum of A-Factor (300 MHz, CDCl<sub>3</sub>) with Literature Data



We assigned these peaks to 2,3-*trans*-A-factor **1.1a**, as shown in Figure 5.2, by comparison with the unambiguously assigned  $^{13}\text{C}$  spectrum of TBDMS protected 2,3-*trans*-A-factor **4.35** (see Appendix Bi and Bii). This assignment revealed that Kinoshita and Hirano had incorrectly assigned C8 and C10, as shown in Figure 5.2. Neither research group, however, mentions the presence of any other resonances, whereas in our spectrum, three additional sets of peaks are apparent, with peak heights approximately 40%, 40% and 8% of the major component. The smallest set of peaks, although partially obscured in the upfield region, is assigned as the enol tautomer **1.1b**, by its close correspondence to the resonances observed for the enol form of TBDMS-protected 2,3-*trans*-A-factor **4.35**, as shown in Table 5.1. The two larger sets of 'extra' peaks were very similar in chemical shift values, suggesting that they represented closely related molecules, while their significant difference from the peaks of 2,3-*trans* A-factor **1.1a** militated against either of them representing the 2,3-*cis* isomer.

**Table 5.1** Comparison of  $^{13}\text{C}$  NMR Data of Enol Tautomers **4.36** and **1.1b**



Enol form	Chemical Shift $\delta$ (ppm)						
	C1	C2	C3	C4	C5	C6	C7
<b>4.36</b> R = TBDMS	173.2*	96.0	39.3	70.0	64.8	176.8*	32.7
<b>1.1b</b> R = H	173.7*	95.5	†	70.0	64.4	176.6*	32.9

† not resolved

\* denotes interchangeable assignments.

Thus the structure of A-factor in solution was found to be significantly more complex than had been assumed by previous workers in this field, and we sought to clarify the situation.

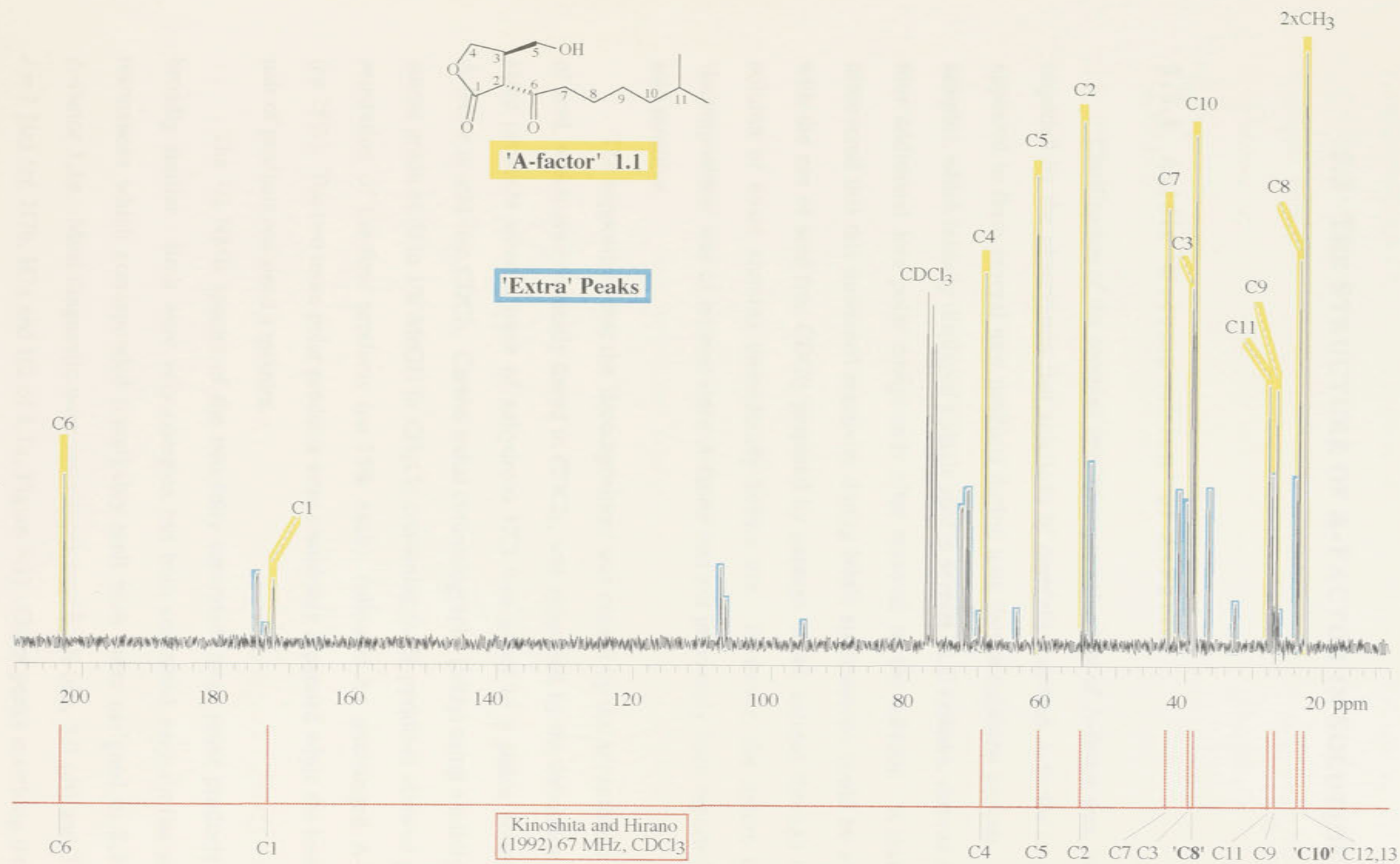


Figure 5.2 Comparison of  $^{13}\text{C}$  NMR Spectrum of A-Factor (50 MHz,  $\text{CDCl}_3$ ) with Literature Data



### 5.3 THE STRUCTURE OF A-FACTOR IN SOLUTION

#### 5.3.1 Acidic 'Decomposition' of A-Factor

Clarification of the complex spectroscopic behaviour of A-factor was, initially, impeded by the observation that solutions of carefully purified A-factor in  $\text{CDCl}_3$  appeared to form several new products during long data acquisition periods. These samples, which initially displayed a single spot in several TLC systems, showed at least four additional less polar compounds after removal of the solvent. It was soon discovered that this unwanted reactivity during NMR spectroscopy could be avoided with the use of acid-free  $\text{CDCl}_3$ , prepared by passage of the solvent through a short column of basic alumina immediately before use. However, the nature of this 'decomposition' was of interest since A-factor had not previously been reported to be acid sensitive.

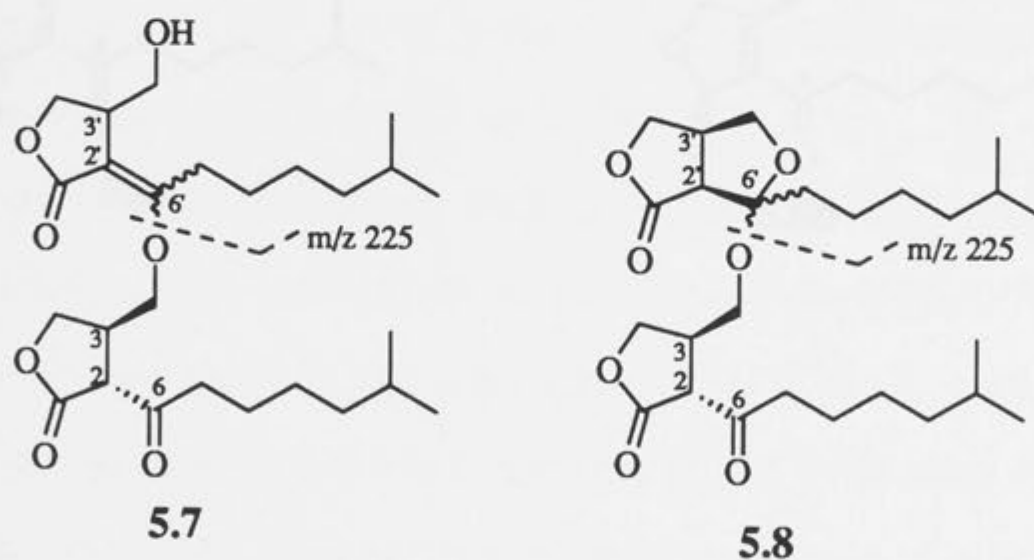
The supposition that the 'decomposition' was caused by the adventitious traces of acid, which are commonly found in  $\text{CDCl}_3$ , was supported by the formation of the same products when a trace of anhydrous  $\text{HCl}$  was added to a stable solution of A-factor in acid-free  $\text{CDCl}_3$ . Careful radial chromatography ( $\text{SiO}_2$ ) using a mildly basic eluent gradient (0 to 1%  $\text{MeOH}$  in  $\text{CH}_2\text{Cl}_2$  containing 0.1% pyridine) allowed partial separation of the four products (*ca* 15% each), followed by unchanged A-factor (*ca* 28%). The two more polar products were completely separated while the less polar pair of products remained a mixture.

The  $^1\text{H}$  NMR spectra of the two fully separated, more polar products were broadly similar. Both were very complex but both contained very similar sets of resonances which corresponded remarkably well with those assigned to 2,3-*trans* A-factor **1.1a**. Most diagnostic were resonances near  $\delta$  2.6 (dt), 3.0 (dt) and 3.6 (d,  $J = 7$  Hz) (*cf.* H7b, H7a and H2 of **1.1a**, Figure 5.1). Other peaks matching those of



2,3-*trans* A-factor **1.1a** were also observed, although the peaks matching the H5a and H5b resonances appear more widely separated in the new products, suggesting some structural difference at C5. Both new products also displayed many additional resonances, suggesting that they were considerably more complex than A-factor, although each apparently incorporated a reasonably intact 2,3-*trans* A-factor moiety. The  $^1\text{H}$  NMR spectrum of the other two less-polar products (combined) showed broadly the same characteristics, suggesting that all four may be closely related.

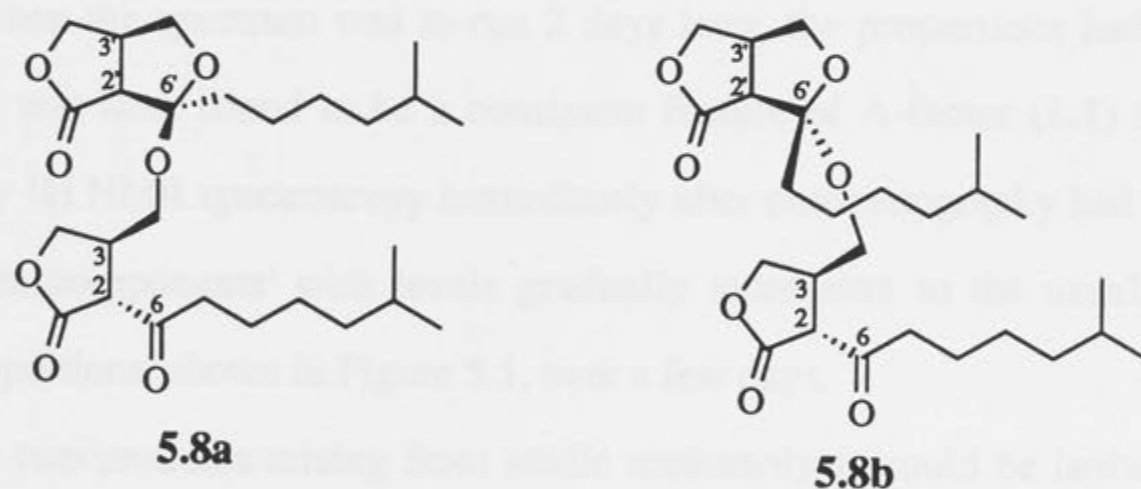
That the relationship of all four compounds was indeed very close (probably diastereomeric) was shown by EIMS and CIMS which displayed identical fragmentation patterns for all product fractions. All products displayed a large  $\text{M}+\text{NH}_4^+$  ion at  $m/z$  484 in their CI mass spectra, indicating a common molecular weight of 466, while the base peak for all compounds, in both the EI and CI mass spectra, was  $m/z$  225. A molecular weight of 466 corresponds to a dimer of A-factor (MW 242) minus a molecule of water. Consideration of plausible dimeric structures of A-factor incorporating a largely intact 2,3-*trans* A-factor moiety and an overall loss of  $\text{H}_2\text{O}$  led to the proposal that the new products were stereoisomers of either **5.7** or **5.8**.



Dimer structures of the type **5.7** were thought less likely on the grounds that the indicated cleavage, required to give the base peak ( $m/z$  225) in the EI and CI mass spectra, is not a favoured process since an unstable vinyl cation would be formed. In contrast, the corresponding cleavage of the ketal dimer structures **5.8** would form a heteroatom-stabilised tertiary cation. An APT spectrum of one of the products (the most polar) gave further support to the ketal dimer structure **5.8** (see Appendix C). In



the downfield region ( $> 80$  ppm) there were 4 quaternary peaks ( $\delta$  202.7, 173.1, 171.8, 109.7), and the one at  $\delta$  109.7 is in the range expected for the ketal carbon (C6').<sup>167</sup> The alternative structure **5.7** requires five downfield quaternary peaks including a distinctive olefinic resonance for C2' expected at  $\delta$  ca 96 (cf. Table 5.2, **4.36**, C2 at  $\delta$  96.0) which was clearly absent. The remainder of the APT spectrum was fully consistent with the proposed ketal dimer **5.8**. The observation of four distinct products is consistent with the two possible pairs of diastereomers **5.8a** and **5.8b**, each of which contains a 2,3-*trans* A-factor moiety and a *cis*-fused [5,5]-bicyclic moiety connected at an asymmetric ketal carbon (C6'). A method of predicting the C2'-C6' relative stereochemistry of the dimers **5.8** is proposed below (see Section 5.4.3). These dimers incorporate A-factor in a previously unknown bicyclic ketal form and their facile formation implied that simpler bicyclic ketals should be formed readily using other alcohols.





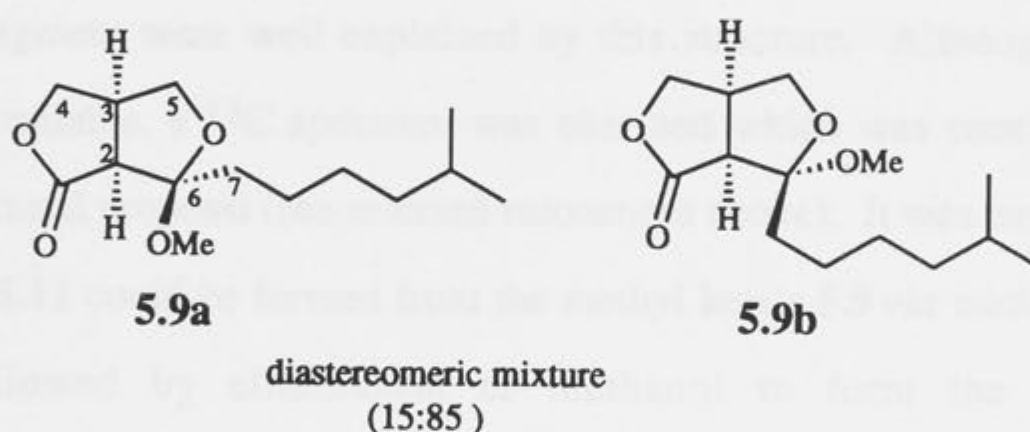
### 5.3.2 Bicyclic Methyl Ketal of A-Factor

Treatment of a  $\text{CDCl}_3$  solution of A-factor with an excess of methanolic HCl (0.25 M) in an NMR tube, allowed ketal formation to be monitored by  $^1\text{H}$  NMR spectroscopy. After 5 minutes the spectrum showed the presence of approximately 70% of two methoxy compounds in a 12:1 ratio (as judged by their methoxyl peaks at  $\delta$  3.16 and 3.22 respectively), and about 30% unreacted A-factor (**1.1**). Strangely, over the following hour the amount of starting material did not appear to decrease significantly (although the ratio of products changed to *ca* 5:1) and after subsequent work-up and chromatography, *ca* 30% of the starting material was recovered. It was interesting to note that the  $^1\text{H}$  NMR spectrum of this recovered A-factor run immediately following chromatography showed a lower than usual proportion of the 'extra' components (representing *ca* 20% of the sample rather than the usual *ca* 40% as in Figure 5.1), although when the spectrum was re-run 2 days later, the proportions had returned to normal. It was later found to be a consistent feature of A-factor (**1.1**) that material analysed by  $^1\text{H}$  NMR spectroscopy immediately after chromatography had lower levels of the 'extra components' with levels gradually increasing to the usually observed relative proportions, shown in Figure 5.1, over a few days.

The two products arising from acidic methanolysis could be isolated, without separation, in a 50% overall yield (15:85 ratio) and were identified by NMR and mass spectroscopy as C6 epimers (**5.9a** and **5.9b**, respectively) of the 2,3-*cis* bicyclic methyl ketal **5.9**. Their molecular weight was determined as 256 by CI mass spectroscopy of the mixture and their EIMS showed the expected major losses at  $\text{M}^+ - \text{OMe}$  ( $m/z$  225) and  $\text{M}^+ - \text{C}_7\text{H}_{15}$  ( $m/z$  157). By  $^1\text{H}$  NMR, their H2 doublets were almost coincident at  $\delta$  3.16 and they displayed  $J_{2,3}$  couplings of 10.9 and 9.2 Hz, for the minor (**5.9a**) and major (**5.9b**) epimers respectively. The vastly reduced ring-strain which would accompany the formation of *cis*-fused rather than *trans*-fused 5-membered rings dictates that these are 2,3-*cis* fused systems even though the coupling constants are within the range expected for 2,3-*trans* substituted  $\gamma$ -lactones.<sup>157</sup> This provided further evidence



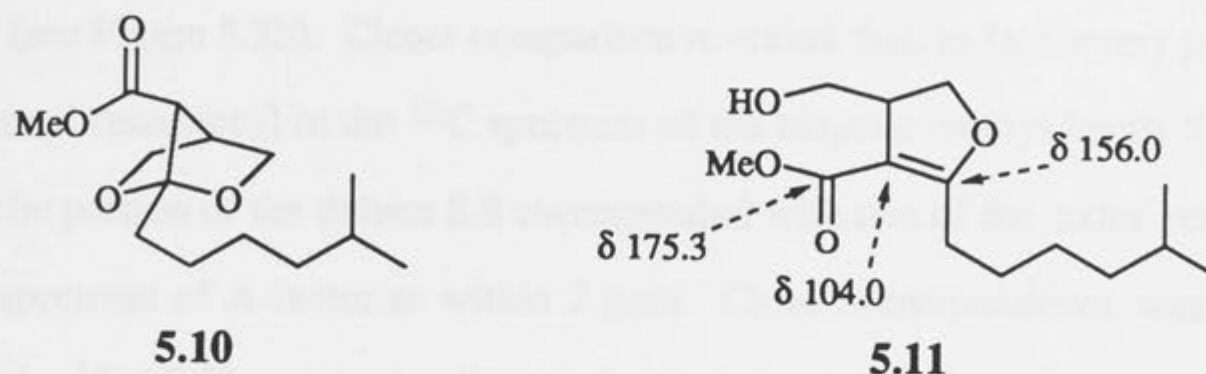
for the lack of general applicability of such empirical correlations, as discussed above (see Section 4.2.3). As expected, the H7 protons were shifted upfield significantly (*ca* 1 ppm) with respect to their position in 2,3-*trans* A-factor **1.1a**, due to the loss of the ketone functionality. Although the relative stereochemistry at C6 could not be determined at this stage, a method for making the indicated assignment was later achieved (this is described in Section 5.4.3). The  $^{13}\text{C}$  NMR (APT) spectrum (see Appendix D) showed the absence of ketone signals and the appearance of the ketal carbons (C6) near  $\delta$  110. The C4 and C5 peaks of the new compounds were shifted downfield and were all nearly coincident around  $\delta$  72. All of the resonances (apart from those due to the methoxyl groups) correlated very well with those assigned to the *cis*-bicyclic portion of the previously isolated A-factor dimers **5.8** (*cf.* Appendix D and Appendix C).



An attempt to fully convert A-factor to its methyl ketals **5.9** by increasing the reaction time to 48 hours, resulted in the isolation of only 40% of the mixed ketals **5.9a** and **5.9b**, (45:55) and a new product (40%) which coincidentally had the same  $R_f$  as the starting material. The new product was shown by EIMS and CIMS to be isomeric with the methyl ketals **5.9** but did not show the same facile loss of  $\text{C}_7\text{H}_{15}$  ( $m/z$  157). Important differences in  $^1\text{H}$  NMR spectra (acid-free  $\text{CDCl}_3$ ) were observed, most notably, the absence of both the H2 doublet at  $\delta$  *ca* 3.2, and the methoxyl resonance, also expected near  $\delta$  3.2. A new three-proton singlet which appeared at  $\delta$  3.74, suggested the presence of a methyl ester. These features, in addition to the observation of generally more complex coupling patterns, led to initial consideration of the novel



[2,2,1]-bicyclic ester structure **5.10**, whose formation *via* methanolysis of the lactone followed by intramolecular ketal formation was considered feasible.



However, re-measuring the  $^1\text{H}$  NMR spectrum in untreated  $\text{CDCl}_3$ , allowed the re-assignment of one doublet of doublets ( $\delta$  2.73, 1H) as a hydroxyl proton due to its collapse to a singlet and change in chemical shift (to  $\delta$  3.8). This clearly eliminated structure **5.10** and the dihydrofuran structure **5.11** was proposed instead. The prominent  $\text{M}^+-\text{OCH}_3$  peak ( $m/z$  225) observed in its EIMS and the absence of a significant  $\text{M}^+-\text{C}_7\text{H}_{15}$  fragment were well explained by this structure. Although only a small sample was available, a  $^{13}\text{C}$  spectrum was obtained which was consistent with this modified structural proposal (see selected resonances above). It was envisaged that the dihydrofuran **5.11** could be formed from the methyl ketals **5.9** *via* methanolysis of the  $\gamma$ -lactone followed by elimination of methanol to form the double bond. Re-lactonisation would then be disfavoured by the excessive ring strain involved with a bridgehead olefin.

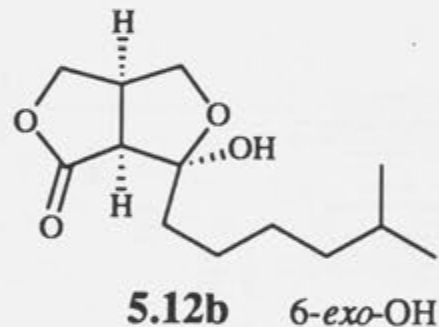
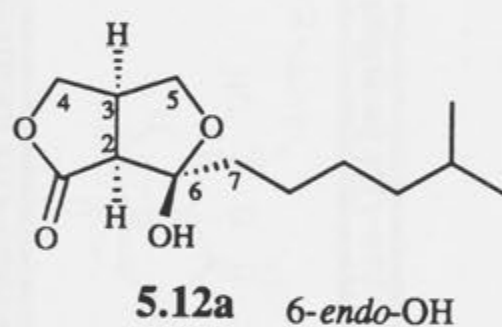
### 5.3.3 Identification of Novel Forms of A-Factor

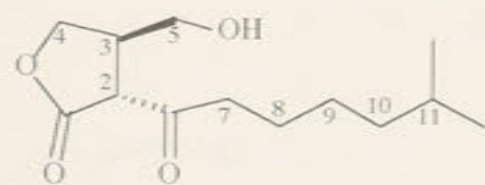
During NMR identification of the bicyclic ketals of A-factor **5.8** and **5.9ab**, striking similarities were noted with the unassigned 'extra' sets of peaks in the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of A-factor, highlighted in Figures 5.1 and 5.2. In particular, it was observed that the H2 resonances of the *cis*-bicyclic ketal moieties appear as doublets between  $\delta$  3.16 and 3.22 ( $J_{2,3}$  *ca* 9 or *ca* 11 Hz), and two such doublets also appeared as minor peaks in the spectrum of A-factor, displaying just such coupling constants (Figure 5.3a). In addition, the  $^{13}\text{C}$  peaks of C4 and C5, which appear grouped around



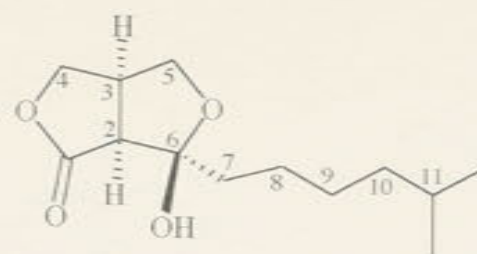
$\delta$  72 in the spectrum of the bicyclic ketals **5.9ab** and the dimers **5.8** (see Appendices C and D), have remarkably similar counterparts as 'extra' peaks in the  $^{13}\text{C}$  spectrum of A-factor (see Figure 5.3b). Closer comparison revealed that, in fact, every peak (except the methoxyl resonance) in the  $^{13}\text{C}$  spectrum of the bicyclic methyl ketals **5.9ab** and in the bicyclic portion of the dimers **5.8** corresponded with one of the 'extra' resonances in the  $^{13}\text{C}$  spectrum of A-factor to within 2 ppm. Close correspondence was also noted between the  $^1\text{H}$  NMR spectra leading to the assignment of the unknown components, which together represent *ca* 35% of A-factor in chloroform solution, as the two diastereomers of the bicyclic hemiketal form **5.12a** and **5.12b**. Thus it became possible to assign all of the 'extra' resonances present in the NMR spectra of A-factor, as shown in Figures 5.3a and 5.3b.

Further support for this assignment comes from the previously noted observation of an otherwise inexplicable band in the IR spectrum of A-factor at  $3606\text{ cm}^{-1}$ , a frequency which is highly characteristic of a tertiary hydroxyl group.<sup>144</sup>

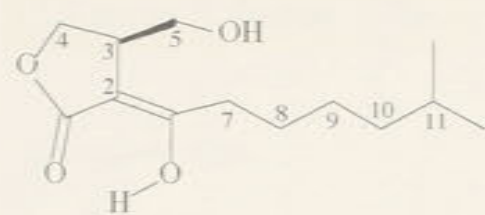




**2,3-trans A-factor 1.1a**

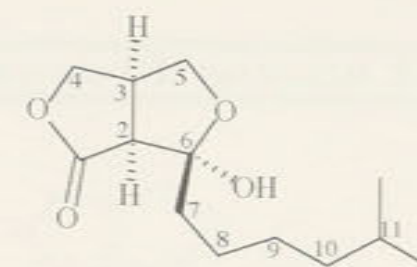


**6-endo-OH hemiketal 5.12a**

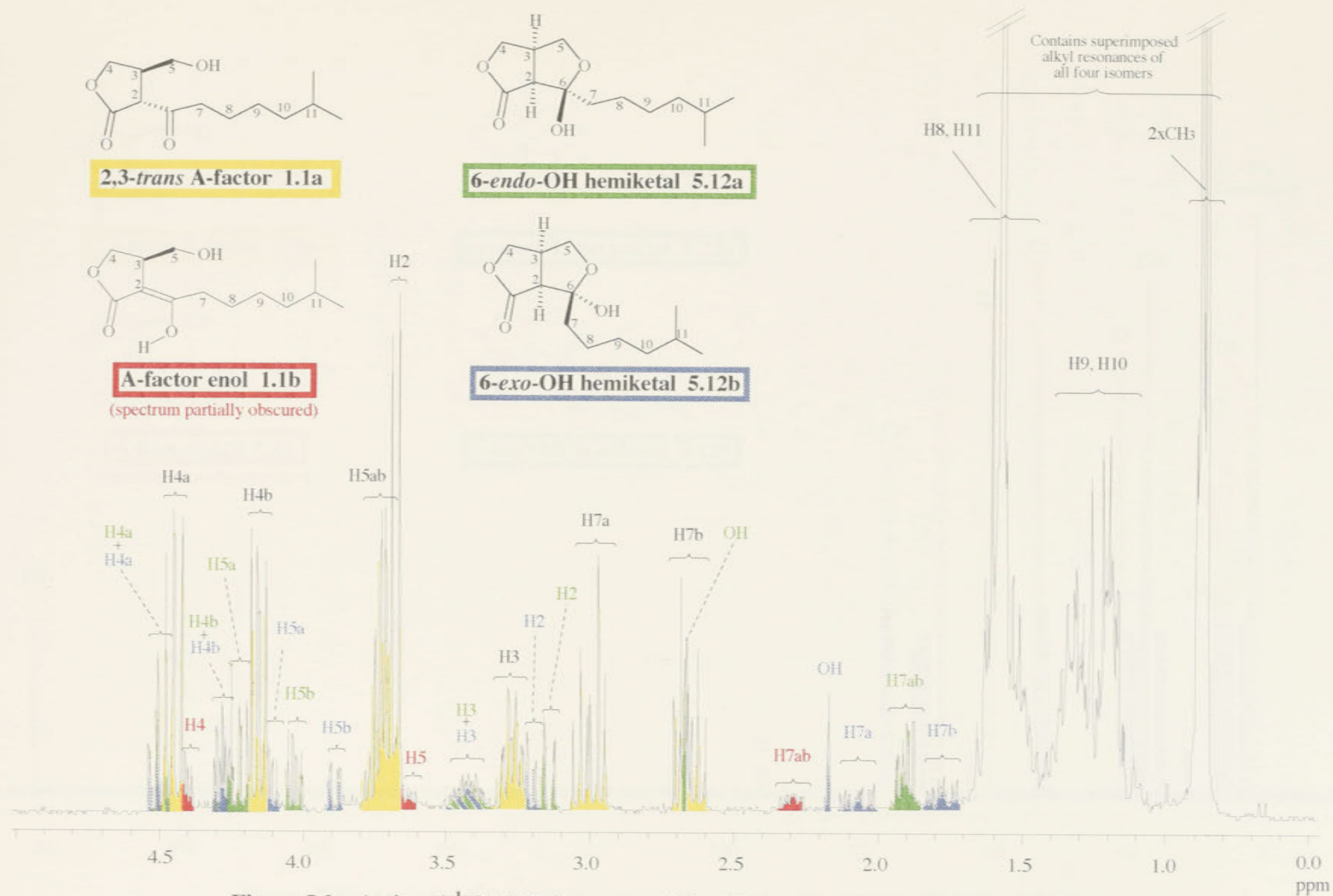


**A-factor enol 1.1b**

(spectrum partially obscured)

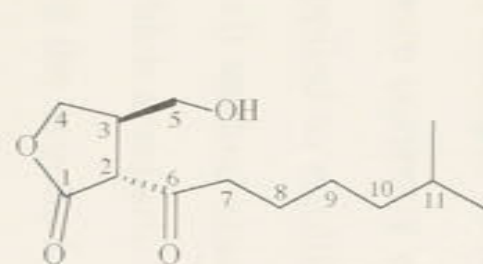


**6-exo-OH hemiketal 5.12b**

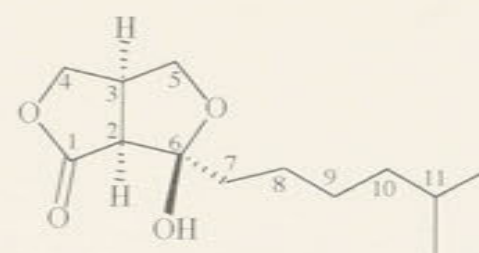


**Figure 5.3a Assigned  $^1\text{H}$  NMR Spectrum of the A-Factor Tautomers (300 MHz,  $\text{CDCl}_3$ )**

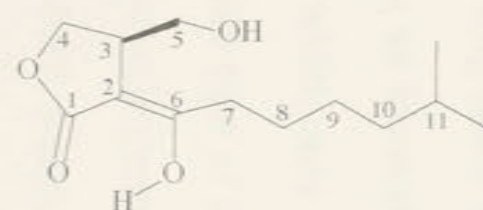




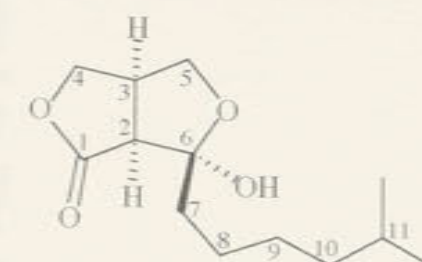
**2,3-trans A-factor 1.1a**



**6-endo-OH hemiketal 5.12a**



**A-factor enol 1.1b**  
(spectrum partially obscured)



**6-exo-OH hemiketal 5.12b**

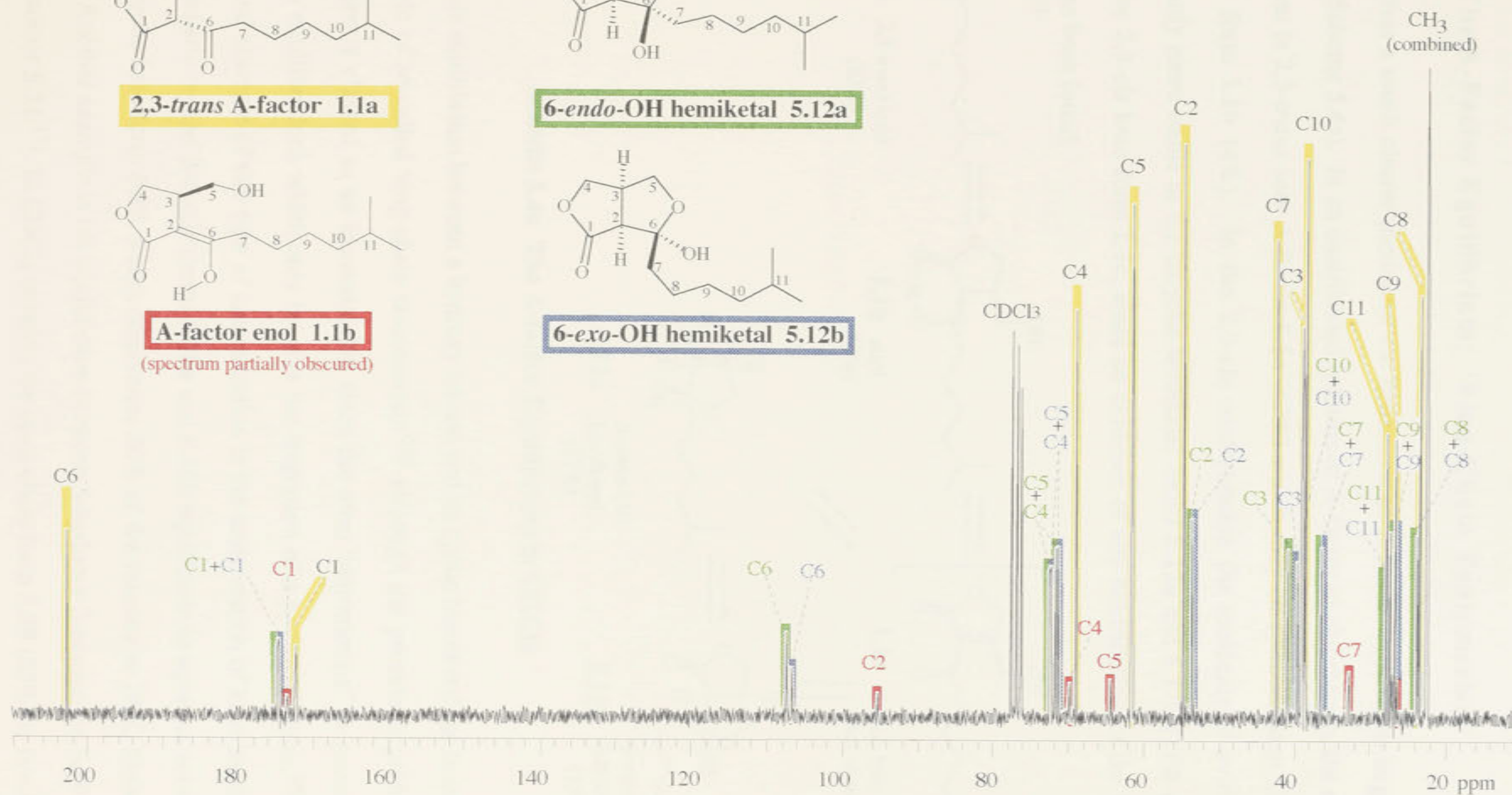
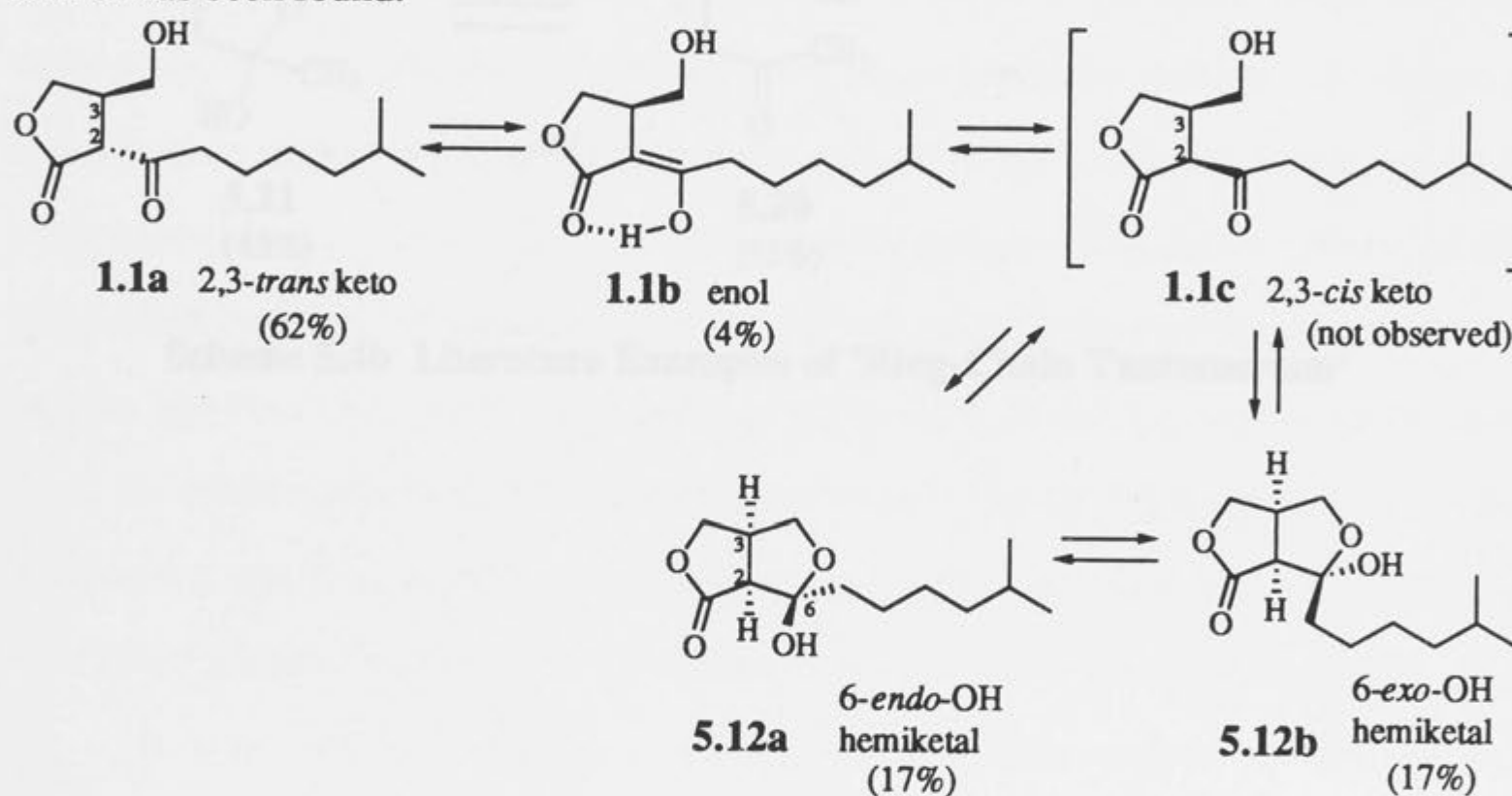


Figure 5.3b Assigned  $^{13}\text{C}$  NMR Spectrum of A-Factor Tautomers (50 MHz,  $\text{CDCl}_3$ )

### 5.3.4 The A-Factor Equilibrium: 'Ring-Chain Tautomerism'

Thus a much clearer picture of the behaviour of A-factor in solution began to emerge (Scheme 5.4a). In an equilibrated solution of A-factor in chloroform, the major component is 2,3-*trans* keto A-factor **1.1a** (62%) which may be epimerised at C2 *via* the enol form **1.1b** (4%). In the 2,3-*cis* configuration the molecule is evidently significantly more stable in the bicyclic hemiketal forms **5.12a** and **5.12b** (17% each) than in the 2,3-*cis* keto form **1.1c**, since no evidence of any contribution of the latter isomer has been found.

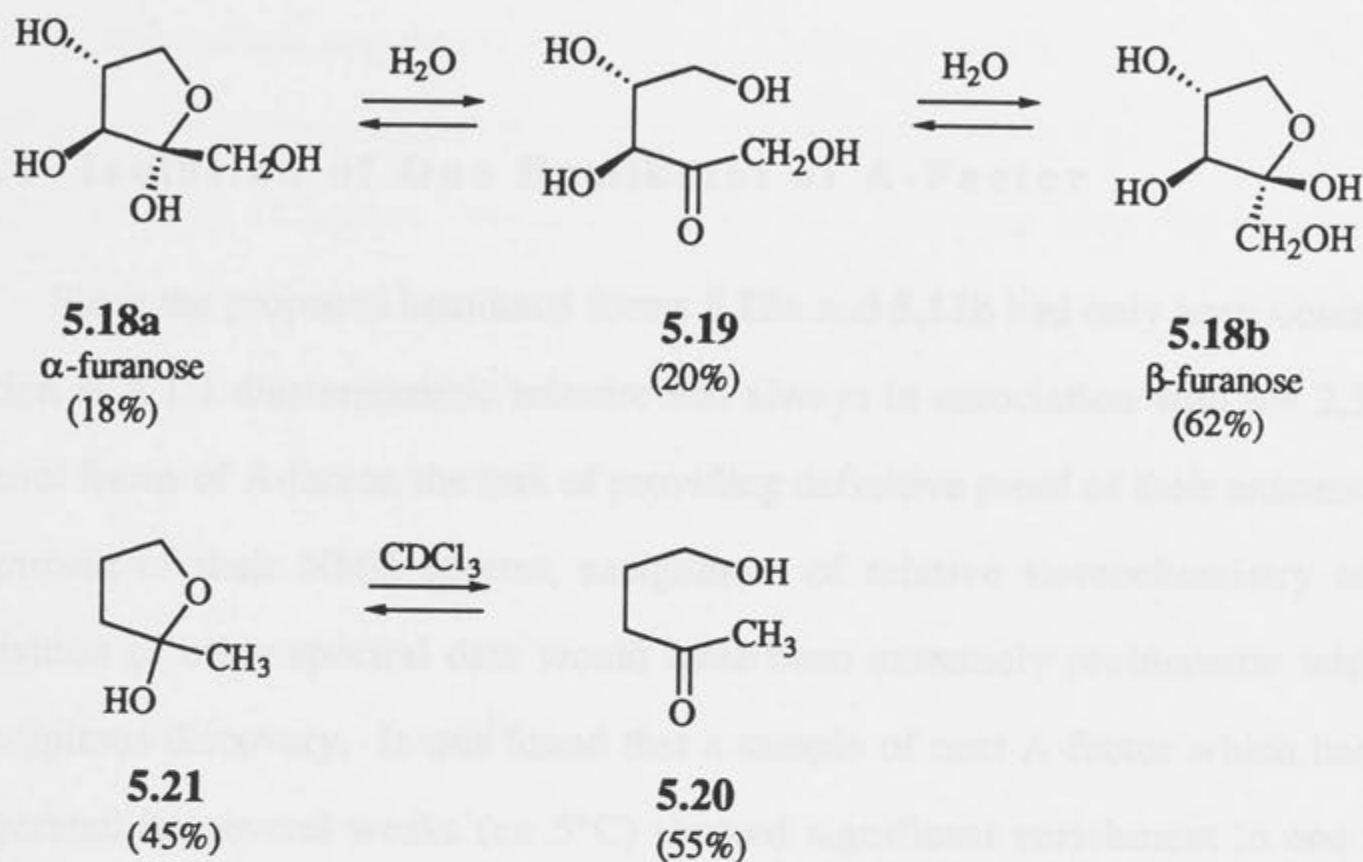


Scheme 5.4a The A-factor Equilibrium in  $\text{CHCl}_3$

This equilibrium between a hydroxy ketone and its cyclic hemiketal (or lactol) is an example of so-called 'ring-chain tautomerism'<sup>168</sup> although the process is probably more correctly classed as an isomerisation since the term 'tautomerism' is generally applied to equilibrations which only involve the migration of a hydrogen atom.<sup>169</sup> A well studied example of this type of isomerisation is the mutarotation of keto sugars in which anomeric  $\alpha$ - and  $\beta$ -cyclic forms **5.18a** and **5.18b** equilibrate in aqueous solution *via* the acyclic keto form **5.19**, which constitutes 20% of the mixture at 26°C (Scheme 5.4b).<sup>170</sup> Another example is the equilibrium between 5-hydroxy-2-pentanone **5.20** and its lactol isomer **5.21**.<sup>171</sup> In  $\text{CDCl}_3$  solution the open chain form **5.20** (55%) is favoured



over the ring form **5.21** (45%), proportions which are analogous to those we observe for A-factor (**1.1**) between its keto **1.1a** and the bicyclic hemiketal forms **5.12** (ratio 65:35).



**Scheme 5.4b Literature Examples of 'Ring-Chain Tautomerism'**

## 5.4 BICYCLIC HEMIKETALS OF A-FACTOR

### 5.4.1 Isolation of One Hemiketal of A-Factor

Since the proposed hemiketal forms **5.12a** and **5.12b** had only been observed in solution as a 1:1 diastereomeric mixture and always in association with the 2,3-*trans* and enol forms of A-factor, the task of providing definitive proof of their existence, full assignment of their NMR spectra, assignment of relative stereochemistry and the acquisition of other spectral data would have been extremely problematic without a serendipitous discovery. It was found that a sample of neat A-factor which had been refrigerated for several weeks (*ca* 5°C) showed significant enrichment in one of the hemiketals, by <sup>1</sup>H NMR spectroscopy. The diastereomeric ratio had changed to *ca* 6:1, and the combined hemiketal forms represented *ca* 78% of the total isomeric mixture. Since the proportion of one diastereomer (characterised by its  $J_{2,3}$  value of 11 Hz) had increased it was then possible to easily distinguish the individual spectra of the two hemiketals which in turn facilitated their assignment as shown above in Figure 5.3.

It was surmised that this enrichment had been caused by the partial crystallisation of one hemiketal, since it had been noted that the sample after storage appeared more solid than the oily wax that characterises 'fresh' samples of A-factor. Crystallisation of one component of the equilibrium mixture could distort the equilibrium in its favour. Many attempts were made to enhance this crystallisation process but only oils were obtained. Eventually, a solution of A-factor in dichloromethane / pentane (*ca* 40:60, *ca* 50 µL/mg) was cooled at -5°C overnight, and we were rewarded with beautiful colourless plates, melting at 69-72°C. The crystals were immediately submitted for X-ray crystallographic analysis (see below). It was later found that this crystallisation could also be accomplished in neat carbon tetrachloride (*ca* 20 µL/mg). An infrared spectrum of the crystalline product in the solid phase (KBr disc) confirmed the lack of a ketone carbonyl absorption and showed



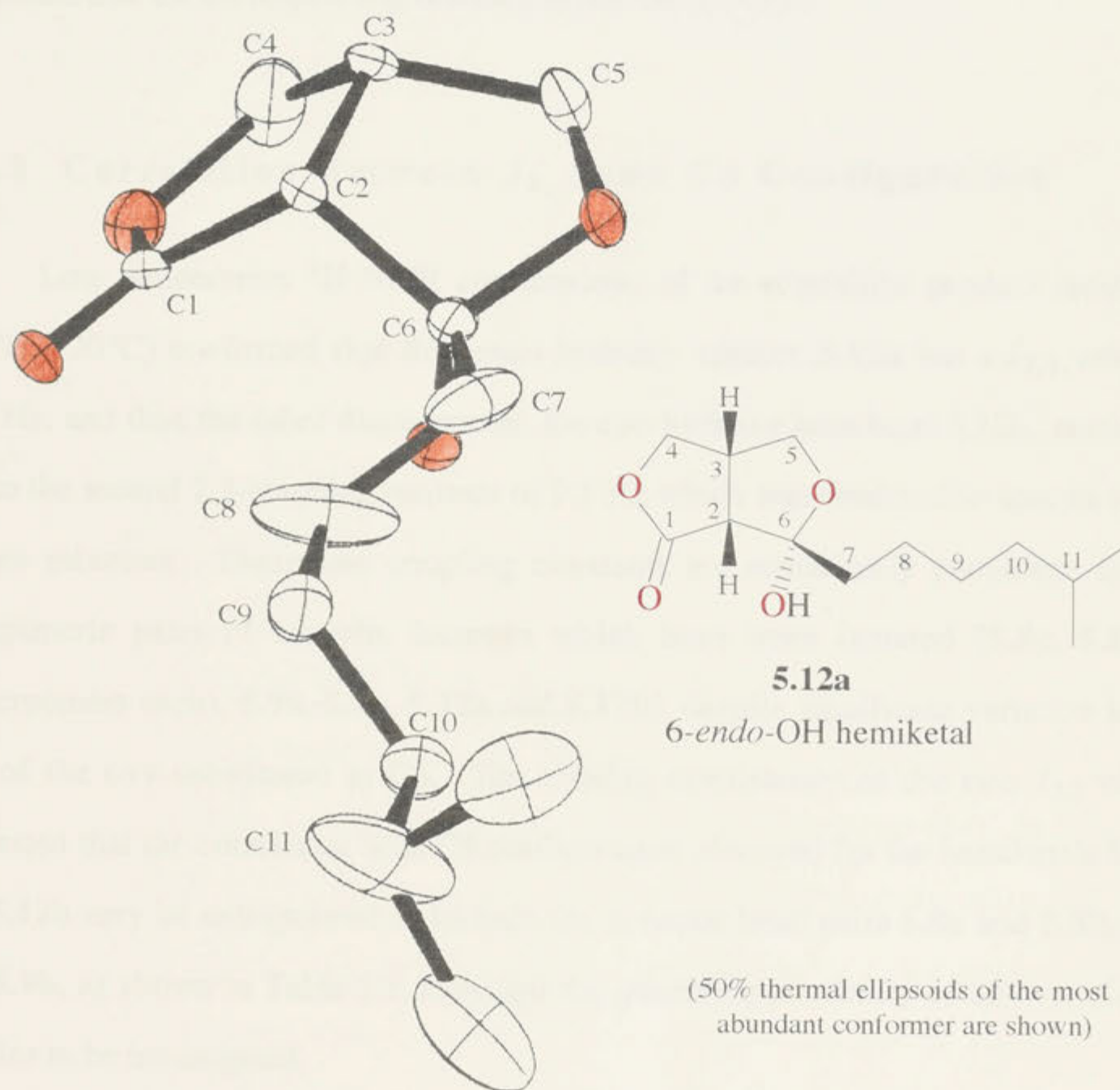
that the lactone carbonyl stretching band had shifted to  $1743\text{ cm}^{-1}$ , presumably as a result of intermolecular hydrogen bonding in the solid phase. The tertiary hydroxyl stretching absorption appeared at  $3395\text{ cm}^{-1}$ , a frequency expected with the influence of strong intermolecular hydrogen bonding.<sup>103</sup> In the previously discussed spectrum of A-factor (**1.1**) in  $\text{CCl}_4$  (see Section 5.2.1) the 'free' OH stretching absorption of this tertiary hydroxyl group and the one of the alternative bicyclic diastereomer **5.12b** are presumably responsible for the otherwise inexplicable band at  $3606\text{ cm}^{-1}$ .

Slow evaporation of a solution of A-factor in dichloromethane : pentane (1:1) at  $-5^\circ\text{C}$ , resulted in the deposition of a fine amorphous powder which contained both hemiketals **5.12a** and **5.12b** in a 55:45 ratio with no associated *trans*-keto A-factor **1.1a**, as judged by  $^1\text{H}$  NMR spectroscopy.

The corresponding 11-demethylhemiketal **5.22a** crystallised from solutions of 11-demethyl A-factor (**3.36**) in cold carbon tetrachloride / hexane. On standing, NMR solutions of **5.22a** formed a mixture containing *trans*-11-demethyl A-factor (**3.3.6**) and the *exo*-hydroxy diastereomer (**5.22b**) as well as **5.22a**. (These structures were assigned by comparison with the NMR spectra of **5.12**.)

#### 5.4.2 X-Ray Structural Analysis

Concerns that the crystals were too thin for X-ray crystal structure analysis proved unfounded and within two days the structure had been solved by Jonathon White (see Experimental, title page) as the *endo*-hydroxy bicyclic hemiketal isomer of A-factor **5.12a** (Figure 5.4). When the X-ray reflection data were recorded at room temperature a satisfactory structure solution could only be obtained for the rigid, bicyclic ring moiety since the flexible alkyl chain exhibited some packing disorder which could not be resolved. When the reflections were remeasured at  $-143^\circ\text{C}$  the nature of the disorder became apparent. The alkyl chain was shown to exist in three distinct conformers which have 25%, 25% and 50% relative abundances.<sup>172</sup> Figure 5.4 depicts the structure of the most abundant conformer, while Appendix Ei shows all atom sites detected.



**Figure 5.4 X-Ray Crystal Structure of 6-*endo*-Hydroxy A-Factor Hemiketal (5.12a)**

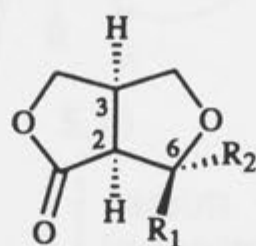


Bond lengths and angles observed in the structure are unexceptional (see Appendix Eii). Strong intramolecular hydrogen-bonding between the hydroxyl and lactone carbonyl groups of **5.12a**, such as is expected in the enol form of A-factor **1.1b**, is precluded by the 3.27 Å separation between the oxygen atoms. For the diastereomeric hemiketal **5.12b**, hydrogen-bonding is also not possible since it was calculated that the corresponding distance would be *ca* 3.8 Å.

#### 5.4.3 Correlation Between $J_{2,3}$ and C6 Configuration

Low temperature  $^1\text{H}$  NMR spectroscopy of the crystalline product (acid-free  $\text{CDCl}_3$ ,  $-20^\circ\text{C}$ ) confirmed that this *endo*-hydroxy epimer **5.12a** has a  $J_{2,3}$  value of 10.8 Hz, and thus the other diastereomer, the *exo*-hydroxy hemiketal **5.12b**, must give rise to the second 2,3-coupling constant of 9.1 Hz which was observed in spectra of the isomer mixtures. These two coupling constants are remarkably consistent for all C6-epimeric pairs of bicyclic lactones which have been isolated [**5.8a**, **5.8b** (2 diastereomers each), **5.9a**, **5.9b**, **5.12a** and **5.12b**], despite significant variation in the size of the oxy-substituent at C6. The striking consistency of the two  $J_{2,3}$  values suggested that the correlation with C6 configuration observed for the hemiketals **5.12a** and **5.12b** may be extrapolated to include the A-factor ketal pairs **5.8a** and **5.8b**, **5.9a** and **5.9b**, as shown in Table 5.2, although the general applicability of this correlation remains to be investigated.

**Table 5.2 Proposed Assignment of C6 Relative Configuration of Bicyclic Ketals Based on  $J_{2,3}$  Values**

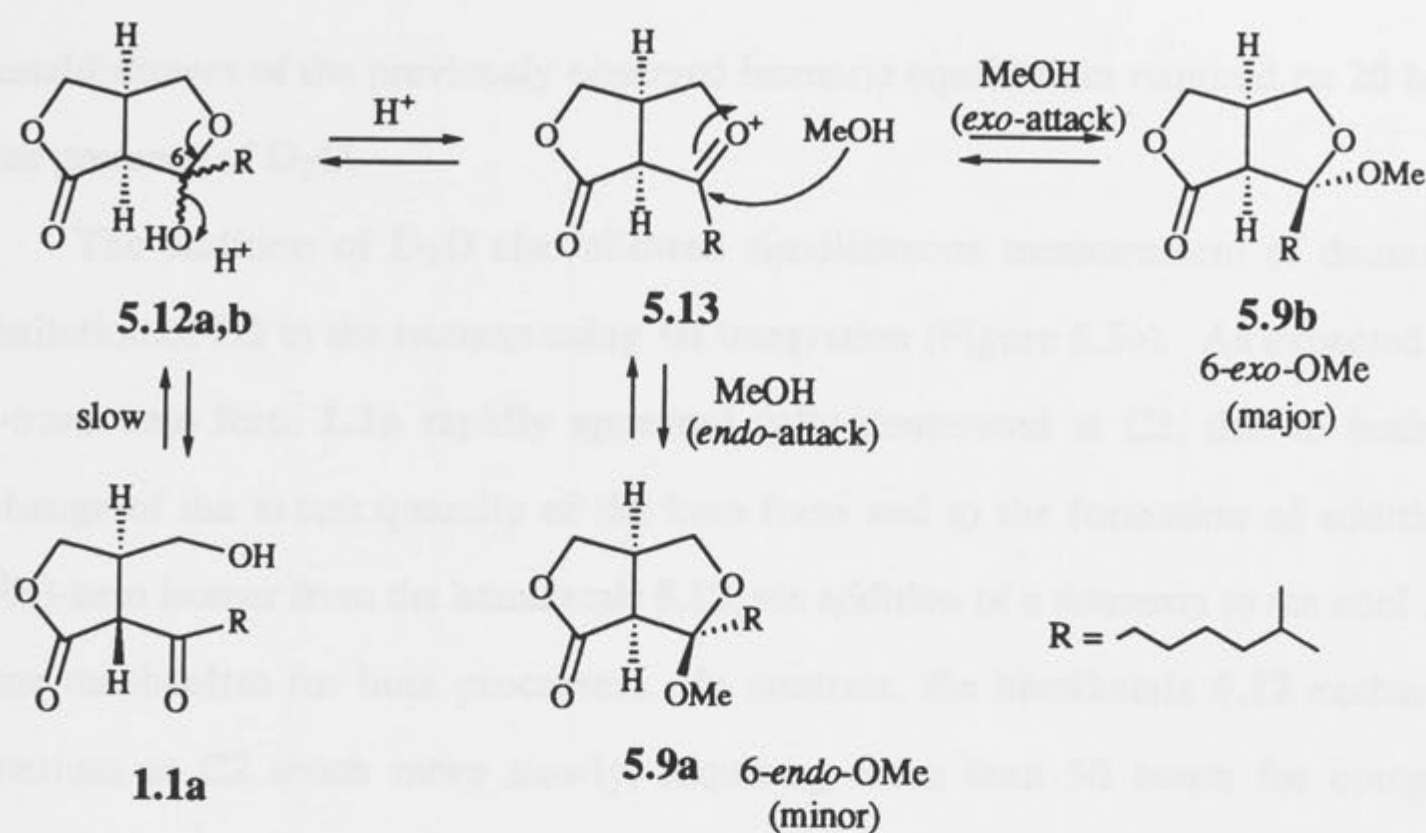


Compound	$J_{2,3}$ value (Hz)	$R_1$ ( <i>endo</i> -substituent)	$R_2$ ( <i>exo</i> -substituent)
<b>5.12a</b>	10.8	OH <sup>†</sup>	(CH <sub>2</sub> ) <sub>4</sub> CH(CH <sub>3</sub> ) <sub>2</sub> <sup>†</sup>
<b>5.9a</b>	10.9	OMe	(CH <sub>2</sub> ) <sub>4</sub> CH(CH <sub>3</sub> ) <sub>2</sub>
<b>5.8a</b> (2 diast.)	11.1, 11.1	<i>O</i> -(2,3- <i>trans</i> A-factor)	(CH <sub>2</sub> ) <sub>4</sub> CH(CH <sub>3</sub> ) <sub>2</sub>
<b>5.12b</b>	9.1	(CH <sub>2</sub> ) <sub>4</sub> CH(CH <sub>3</sub> ) <sub>2</sub> <sup>†</sup>	OH <sup>†</sup>
<b>5.9b</b>	9.2	(CH <sub>2</sub> ) <sub>4</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	OMe
<b>5.8b</b> (2 diast.)	9.0, 9.1	(CH <sub>2</sub> ) <sub>4</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	<i>O</i> -(2,3- <i>trans</i> A-factor)

<sup>†</sup> Assignment supported by X-ray structure.

In the case of the methyl ketals **5.9a** and **5.9b**, this assignment is supported by <sup>1</sup>H NMR observations made during their formation (see Section 5.3.2). The A-factor sample used as starting material was noted to be significantly enriched in the hemiketal isomers **5.12a** and **5.12b**, (**1.1a** : **5.12a** : **5.12b**, 28:44:28%) presumably by partial crystallisation during storage. Within 5 minutes of the addition of the methanolic HCl, the hemiketals had been almost entirely converted into a mixture of bicyclic methyl ketals **5.9** in which the epimer exhibiting a 9.2 Hz 2,3-coupling constant dominated by a ratio of 12:1, while the 2,3-*trans* keto A-factor remained largely unchanged. Assignment of the kinetically favoured epimer as the *exo*-methoxy ketal **5.9b** is consistent with its formation *via* preferential attack by methanol from the less hindered *exo*-face of the intermediate oxonium ion **5.13** (Scheme 5.5). Over the following hour, some equilibration between the epimers was observed, reducing the ketal epimer ratio to *ca* 6:1. The quantity of the 2,3-*trans* keto A-factor **1.1a** remained almost undiminished over this period.





Scheme 5.5

#### 5.4.4 Isomerisation Rate

The observation that the keto form of A-factor **1.1a** was not appreciably consumed after 1 hour in the presence of methanolic HCl (see above) shows firstly that methyl ketal formation occurs largely (or exclusively) through the bicyclic hemiketal isomers **5.12a** and **5.12b** and secondly, that the keto-hemiketal conversion (**1.1a** to **5.12**) is surprisingly slow under these conditions.

Investigation of the rate of isomerisation was made possible by the isolation of the crystalline hemiketal **5.12a**. A sample of this isomer in acid-free  $\text{CDCl}_3$ , showed by NMR that equilibration (to a ratio of *ca* 1:1) with its C6 epimer **5.12b** was relatively rapid (*ca* 12 h), but isomerisation to the 2,3-*trans* keto isomer **1.1a** proceeded much more slowly; reforming the previously observed equilibrium mixture (**1.1a** : **1.1b** : **5.12a** : **5.12b**, 62:4:17:17%, see Scheme 5.4a) only after *ca* 3 days at room temperature.

Further investigation showed that these equilibration processes are both apparently accelerated by the presence of water. The isomerisation of a solution of the crystalline A-factor isomer **5.12a** in acid-free  $\text{CDCl}_3$  was monitored by  $^1\text{H}$  NMR spectroscopy for 9.5 hours before the addition of  $\text{D}_2\text{O}$ . Figure 5.5a illustrates that the rate of hemiketal-keto isomerisation established during this initial equilibration period was significantly increased after the  $\text{D}_2\text{O}$  addition. Under these conditions,

re-establishment of the previously observed isomeric equilibrium required *ca* 20 hours in the presence of D<sub>2</sub>O.

The addition of D<sub>2</sub>O also allowed simultaneous measurement of deuterium substitution of H<sub>2</sub> in the isomers using <sup>1</sup>H integration (Figure 5.5b). As expected, the 2,3-*trans* keto form **1.1a** rapidly appeared fully deuterated at C2, due to both H<sub>2</sub> exchange of the extant quantity of the keto form and to the formation of additional [2-<sup>2</sup>H]-keto isomer from the hemiketals **5.12**, *via* addition of a deuteron to the enol **1.1b** (same mechanism for both processes). In contrast, the hemiketals **5.12** exchanged deuterium at C2 much more slowly, requiring more than 50 hours for complete deuteration, presumably because they need to be first converted to the 2,3-*cis* keto form **1.1c** before exchange at C2 can occur. (The hemiketal itself is presumably not acidic as the conjugate base is a bridgehead enolate.) Since this deuteration and the equilibration with **1.1a** both presumably require the enol form **1.1b** as an intermediate (see Scheme 5.4a), it is not surprising that they occur over a similar time frame. It appeared likely that in these processes the rate determining step was the ring-opening of the bicyclic ring system to form the 2,3-*cis* keto isomer **1.1c**.



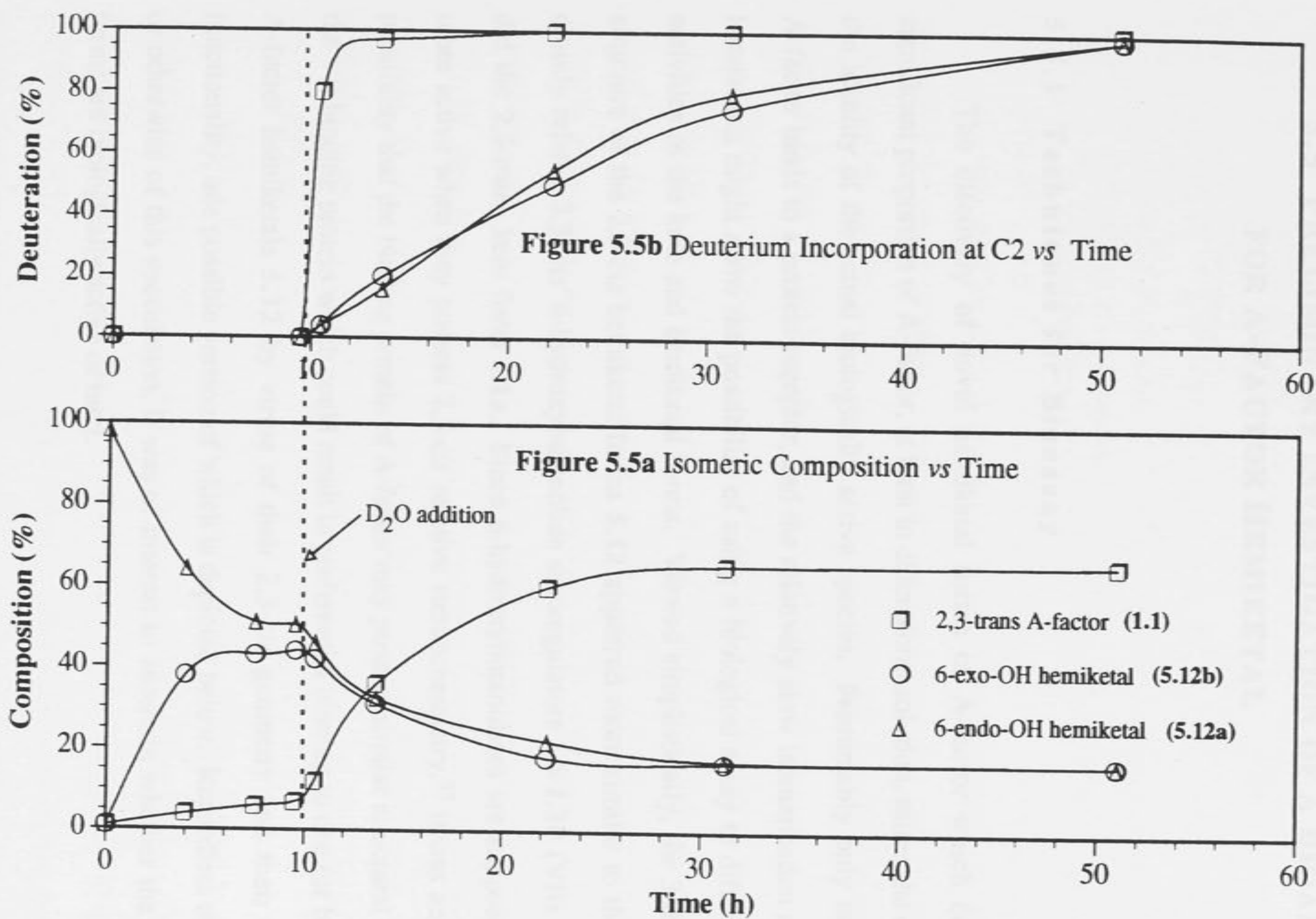


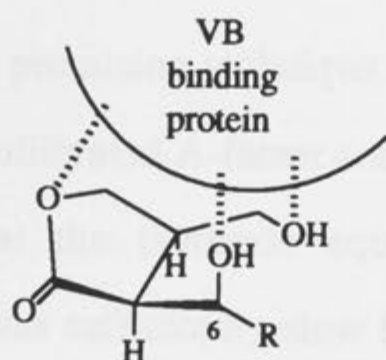
Figure 5.5 Effect of D<sub>2</sub>O on the Equilibration of the 6-*endo*-OH Hemiketal (5.12a) in CDCl<sub>3</sub>

## 5.5 PRELIMINARY INVESTIGATION OF A BIOASSAY FOR A-FACTOR HEMIKETAL

### 5.5.1 Techniques for Bioassay

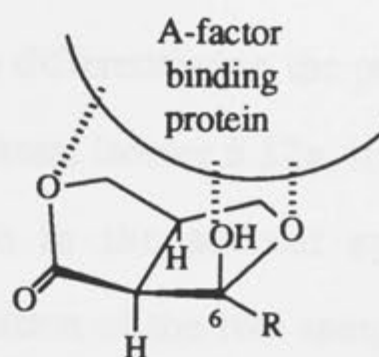
The discovery of novel hemiketal forms of A-factor which constitute a significant proportion of A-factor, at least in chloroform solution, raises the question of the identity of the actual biologically active species. Presumably only one form of A-factor binds to a protein receptor, and the relatively slow isomerisation rate for the hemiketals might allow the possibility of using a biological assay to differentiate the activities of the keto and hemiketal forms. Viewed simplistically, the 3-dimensional structure of the 2,3-*cis* hemiketal forms **5.12** appeared more similar to the series of closely related 2,3-'*cis*' 6-hydroxybutanolide autoregulators **1.6-1.10** (VBs A-E) than did the 2,3-*trans* keto form **1.1a**. Since 6-hydroxybutanolides are supposedly much more active when they possess 2,3-*cis* relative stereochemistry,<sup>35</sup> it was an intriguing possibility that the binding protein of A-factor may possess similar structural features to the VB binding protein which could result in preferential binding to one (or both) of the A-factor hemiketals **5.12** by virtue of their 2,3-*cis* geometry and their 6-hydroxy functionality, one possible version of which is depicted below. Regardless of the merit or otherwise of this speculation, it was of interest to ascertain whether the hemiketal forms are biologically active or inert.



**1.6-1.10** VBs A-E

2,3-'cis' 6-OH autoregulators

R = various alkyl

**5.12a** hemiketal A-factor

R =

If a biological assay for A-factor existed which could be completed substantially faster than the period required for hemiketal / keto equilibration, then it would be very interesting to compare the relative activities of the pure hemiketal **5.12a** with that of a sample of A-factor which had been allowed to reach equilibrium.

A-factor and analogues have been previously been bioassayed by plate assay,<sup>14</sup> in which a plate culture of an A-factor deficient mutant of *Streptomyces griseus* is treated with a spot sample of the compound to be tested. After further incubation for 2 days, the induction of streptomycin production in this location is tested by subsequent introduction of a culture of *Bacillus subtilis*, and measurement of the diameter of the inhibition zone around the sample site. It was considered likely that during the time required for this test, a sample of A-factor hemiketal **5.12a** would largely (or completely) revert to the usual mixture of A-factor isomers, making detection of any activity difference unlikely.

Fortunately, around this time an A-factor protein-binding assay was reported by Miyake *et al.*<sup>49</sup> which only required the A-factor to be dissolved for less than 2 hours. The binding assay involved the preparation of a crude, cell-free protein extract from a culture of a streptomycin-producing strain of *S. griseus* followed by the addition of a sample of radioactively labelled [<sup>3</sup>H]-A-factor. Incubation for 90 minutes, followed by Sephadex G-25 chromatography, allowed separation of the receptor-bound [<sup>3</sup>H]-A-factor from the unbound compound. Peaks of radioactivity due to these two forms were detected by liquid scintillation counting of the chromatographic fractions.



For this promising technique to be of use in differentiating the protein-binding efficacies of equilibrated A-factor and its pure hemiketal isomer **5.12a**, it had first to be established that the isomeric equilibration rate in the solvent system of the binding-assay was sufficiently slow for the composition of the two samples to remain significantly different for the duration of the test (*ca* 2 hours). Secondly, an efficient method for the preparation of tritium-labelled A-factor hemiketal had to be developed.

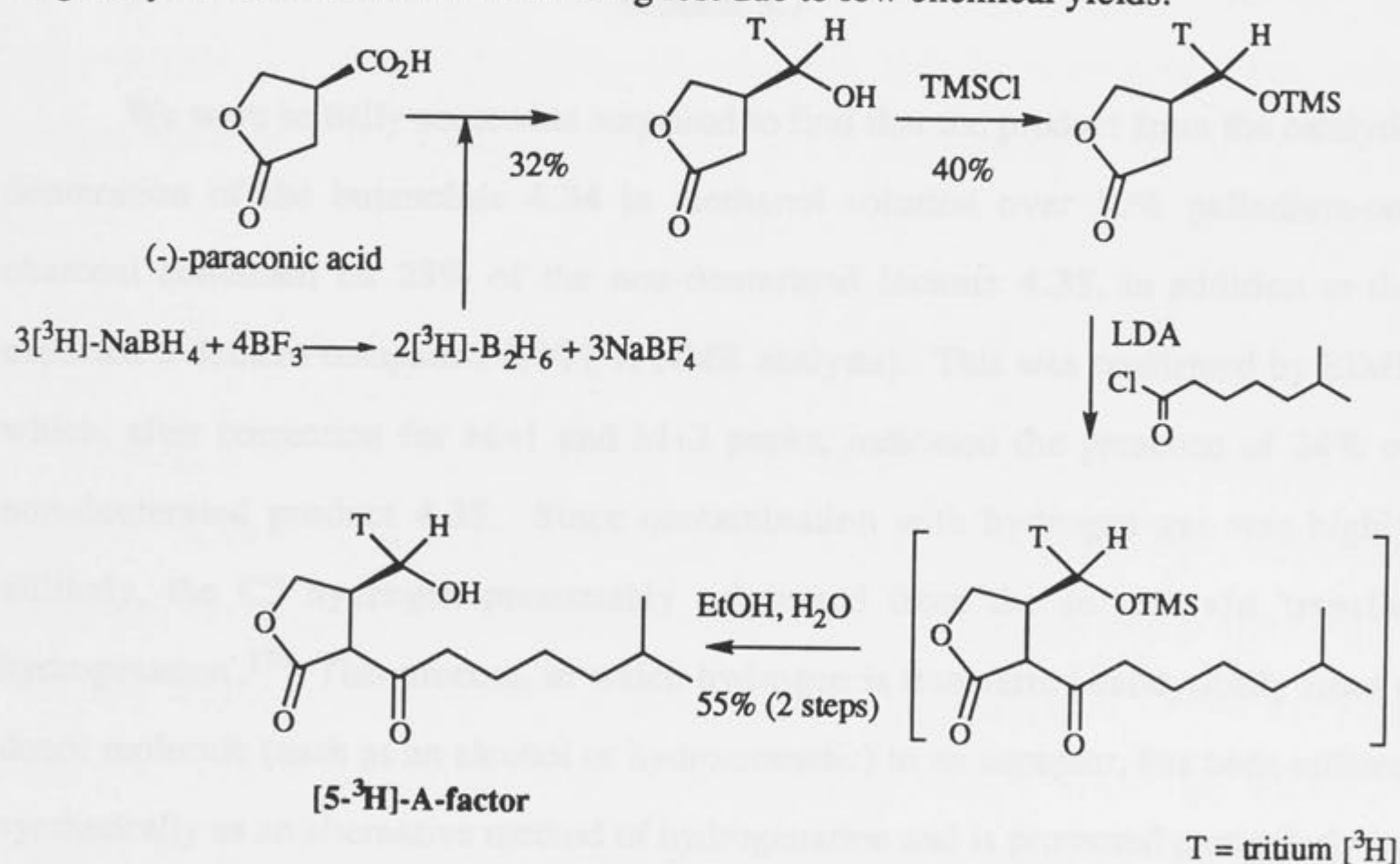
The required equilibration rate could have been easily measured by  $^1\text{H}$  NMR if the binding-assay solvent system could have been replicated in fully deuterated form. Unfortunately, the assay requires a rather complex buffer system to stabilise the proteins, and its components are not readily available as fully deuterated compounds. [Buffer system: triethanolamine (50 mM), KCl (0.5 M), NaCl (0.15 M), phenylmethylsulfonyl fluoride (0.1 mM), EDTA (5 mM), 2-mercaptoethanol (5 mM), glycerol (10% v/v), pH 7.0]. Rather than try to approximate this system with available (but expensive) deuterated materials it was considered that an inverse approach may be better, in which the isomerisation of *deuterium-labelled* A-factor hemiketal **5.12a** was observed, by *deuterium* NMR, using the normal, non-deuterated buffer mixture as a solvent. An extra benefit of this approach is that the technique used to prepare  $[^2\text{H}]$ -A-factor could later be applied to the synthesis of the tritium-labelled material required for the protein-binding study.

It was recognised that, in view of the acceleration of equilibration observed when  $\text{D}_2\text{O}$  was added to a  $\text{CDCl}_3$  solution of A-factor hemiketal (Figure 5.5a) the largely aqueous buffer mixture may increase further the rate of isomerisation, although the magnitude of any effect could not be predicted due to the complexity of the system. It was felt that the neutrality of the buffer would at least minimise any increase in the isomerisation rate.

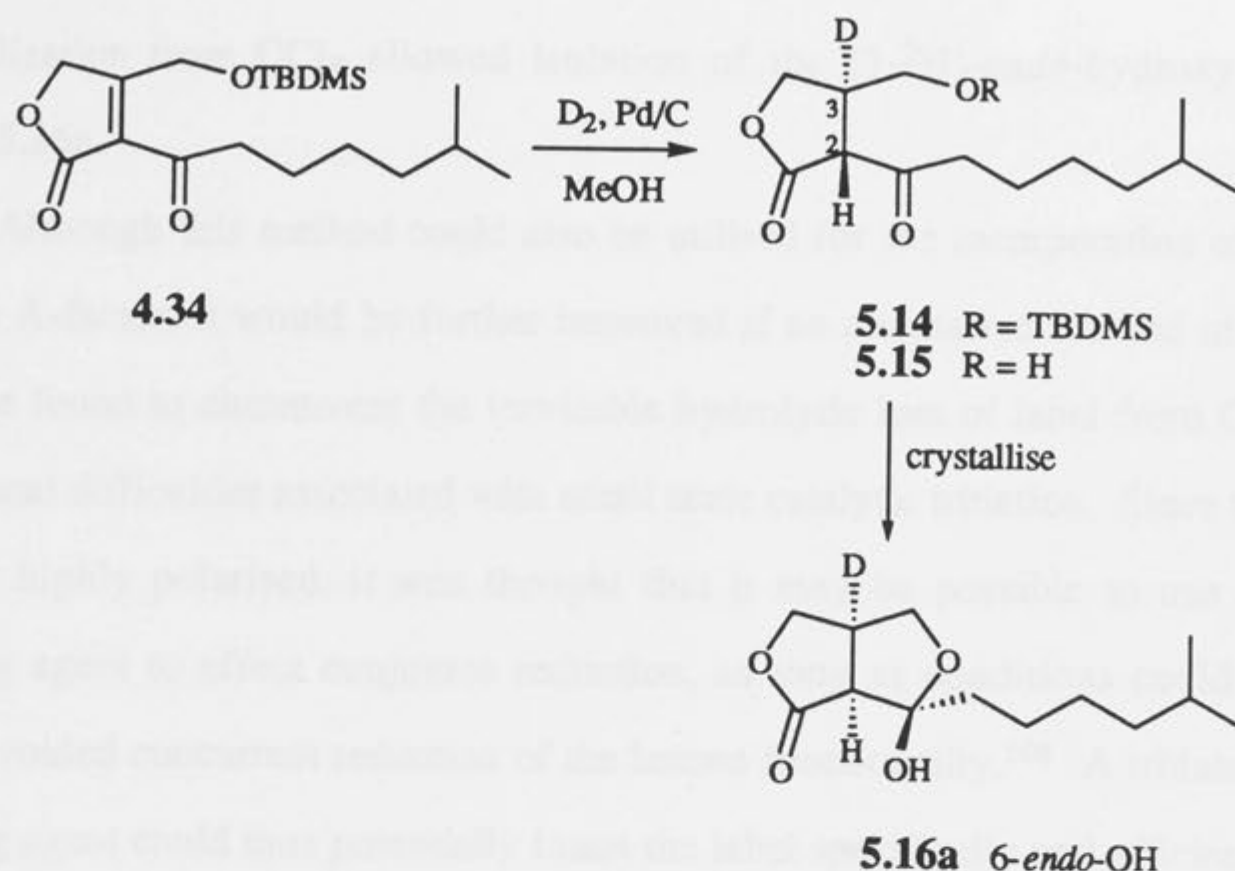


5.5.2 Synthesis of [ $^2\text{H}$ ]-A-Factor Hemiketal

The only previous report of the preparation of isotopically-labelled A-factor was by Miyake *et al.*<sup>49</sup> who introduced a tritium label at C5 using a route whose complexity and inefficiency left considerable scope for improvement (Scheme 5.6). It was based on Mori's asymmetric synthesis from (-)-paraconic acid, with the label being inserted *via* a low yielding reduction of the acid function using [ $^3\text{H}$ ]-diborane; a reagent which had to be prepared from [ $^3\text{H}$ ]- $\text{NaBH}_4$ . Since the label was introduced early in the synthesis, the remaining moderate yielding steps cause extensive losses of costly and hazardous tritiated materials. An overall yield of 7% was obtained, with 78% of the originally introduced tritium label being lost due to low chemical yields.

Scheme 5.6 Synthesis of [ $5\text{-}^3\text{H}$ ]-A-Factor by Miyake *et al.*

Fortunately, an additional feature of our synthetic route is that it allows the easy introduction of a C3 label at a late stage of the synthesis, during the reduction of the butenolide intermediate **4.34**. Substitution of deuterium for hydrogen in the usual catalytic hydrogenation procedure provided a simple method of generating the required [ $3\text{-}^2\text{H}$ ]-A-factor **5.15** (Scheme 5.7).



Scheme 5.7

We were initially somewhat surprised to find that the product from the catalytic deuteration of the butenolide **4.34** in methanol solution over 10% palladium-on-charcoal contained *ca* 28% of the non-deuterated lactone **4.35**, in addition to the expected 3-deutero compound **5.14** ( $^1\text{H}$  NMR analysis). This was confirmed by EIMS which, after correction for  $M+1$  and  $M+2$  peaks, indicated the presence of 24% of non-deuterated product **4.35**. Since contamination with hydrogen gas was highly unlikely, the C3 hydrogen presumably originated from the solvent *via* 'transfer hydrogenation'.<sup>173</sup> This process, in which hydrogen is transferred catalytically from a donor molecule (such as an alcohol or hydroaromatic) to an acceptor, has been utilised synthetically as an alternative method of hydrogenation and is promoted particularly by palladium.<sup>173</sup> This result illustrates that the transfer hydrogenation mechanism may account for a surprisingly large amount of the reactivity of catalytic hydrogenations carried out in alcoholic solvents using palladium catalysis. The formation of the unlabelled product **4.35** could presumably be avoided if a non-alcoholic solvent was used (*e.g.* ethyl acetate) but this was not pursued since the level of specific deuterium incorporation achieved was adequate for the purposes of the isomerisation rate study. Desilylation of the lactone **5.14** gave  $[3\text{-}^2\text{H}]$ -A-factor **5.15** in an overall yield of 62%.



Crystallisation from  $\text{CCl}_4$  allowed isolation of the  $[3\text{-}^2\text{H}]\text{-endo}$ -hydroxy hemiketal isomer **5.16a**.

Although this method could also be utilised for the incorporation of a tritium label to A-factor, it would be further improved if an alternative method of reduction could be found to circumvent the inevitable hydrolytic loss of label from C2 and the operational difficulties associated with small scale catalytic tritiation. Since the double bond is highly polarised, it was thought that it may be possible to use a hydride reducing agent to effect conjugate reduction, as long as conditions could be found which avoided concurrent reduction of the ketone functionality.<sup>106</sup> A tritiated hydride reducing agent could thus potentially insert the label specifically and efficiently at C3, with hydrogen addition at C2 occurring through subsequent protonation. This approach was not explored at this stage but a closely related regioselective reduction using sodium borohydride was subsequently accomplished, as described in Chapter 7.

### 5.5.3 $^2\text{H}$ NMR Monitored Study of Isomerisation Rate

High resolution  $^2\text{H}$  NMR spectroscopy is a powerful analytical technique for the study of reaction mechanisms and kinetics, which until recently has not been widely employed due to misconceptions about its sensitivity, resolution and practicality.<sup>174</sup> The low magnetogyric ratio of the deuterium nucleus does lead to low detection sensitivity, (0.96% relative to  $^1\text{H}$  and 66% relative to  $^{13}\text{C}$ ) but when fully deuterated samples are used, sensitivity is in practice more than 50 times greater than for the widely used natural abundance  $^{13}\text{C}$  spectroscopy. In addition, the low sensitivity of  $^2\text{H}$  nucleus is somewhat offset by its rapid relaxation *via* quadrupolar interactions which allows transients to be accumulated more rapidly than with  $^1\text{H}$  or  $^{13}\text{C}$  spectroscopy. Short relaxation times and the lack of nuclear Overhauser enhancement allow accurate integration of deuterium signals. Although the significant electric quadrupole moment of the  $^2\text{H}$  nucleus might be expected to lead to serious line broadening as a result of quadrupolar relaxation, the effects are much less than for other heavier quadrupolar



nuclei such as  $^{14}\text{N}$ ,  $^{11}\text{B}$  and  $^{35}\text{Cl}$  and line widths attained are often comparable to those of  $^1\text{H}$  spectroscopy when measured in Hz. For example, the  $^2\text{H}$  resonance of deuteriochloroform has a width at half height of *ca* 0.5 Hz. Peaks may sometimes appear broadened by inhomogeneities of the external magnetic field or from the incomplete coalescence of multiplets arising from the  $^1\text{H}$ - $^2\text{H}$  couplings but these effects can be minimised by careful magnetic field shimming and by  $^1\text{H}$  decoupling. A very useful feature of  $^2\text{H}$  NMR spectroscopy is that the deuterium chemical shifts are essentially the same as those of the analogous  $^1\text{H}$  isotope, which facilitates the assignments of peaks.

Prior to the commencement of the isomerisation rate study it was necessary to confirm that the  $^2\text{H}$  NMR resonances of the keto and hemiketal isomers **5.15** and **5.16** could be distinguished and quantified. A sample of labelled A-factor was allowed to come to isomeric equilibrium in acid-free  $\text{CHCl}_3$  before running a  $^1\text{H}$  decoupled  $^2\text{H}$  NMR spectrum. The spectrometer was run unlocked since the deuterium lock channel was in use as the observation channel. As in the analogous  $^1\text{H}$  spectrum, two broad resonances were observed at  $\delta$  3.4 and 3.2 representing the C3 deuterium of the combined hemiketals **5.16** and keto isomer **5.15**, respectively, with an integration ratio (34:66) which corresponded well to previous measurements. A crystalline sample of the *endo*-hydroxy hemiketal **5.16a**, dissolved in  $\text{CHCl}_3$ , initially displayed a single peak at  $\delta$  3.4. As a test of the feasibility of monitoring the isomerisation, some of the aqueous buffer mixture required for the protein-binding assay was then added followed by an equal volume of acetonitrile to assist miscibility of the mixture, ( $\text{CHCl}_3$  : buffer :  $\text{CH}_3\text{CN}$ , 4:1:1). The altered solvent composition shifted the peak slightly upfield to  $\delta$  3.3 and caused the appearance of two low intensity peaks at  $\delta$  4.5 and 2.0 attributed to the natural-abundance  $^2\text{H}$  resonances of water and acetonitrile, respectively. Spectra run periodically over the following 8 hours showed the gradual diminution of the peak representing the hemiketals **5.16** and the appearance of a peak at  $\delta$  3.0 representing the keto isomer **5.15**. After 8 hours, the integration ratio of these peaks was *ca* 60:40 and still falling but further monitoring could not be performed due



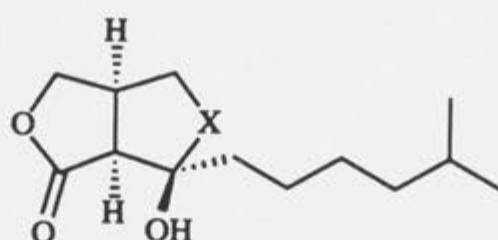
to restricted spectrometer time. It was estimated that more than 12 hours would have been required for full equilibration. This trial successfully demonstrated that kinetic monitoring of the isomerisation by  $^2\text{H}$  NMR was viable but the results lacked relevance to the proposed binding-assay since chloroform was the dominant component of the solvent mixture.

Unfortunately, solubility did not allow a test of isomerisation to be performed using only the binding-assay buffer mixture as the solvent. It was not possible to dissolve sufficient labelled A-factor hemiketal **5.16a** in the buffer mixture for it to be discernible by  $^2\text{H}$  spectroscopy within a reasonable acquisition period. As a compromise, acetonitrile (30%) was added to the buffer mixture to allow dissolution of the required amount of the hemiketal **5.16a**. A trace of  $\text{CDCl}_3$  was added as a  $^2\text{H}$  NMR reference. Surprisingly, a series of  $^2\text{H}$  NMR spectra run immediately following dissolution indicated that under these conditions, equilibration of the hemiketal **5.16a** was essentially complete in less than 10 minutes. The spectrum run after *ca* 10 minutes showed resonances representing the hemiketal and keto isomers at  $\delta$  3.0 and 2.7 respectively, with an integration ratio of 36:64. After 12 hours the isomeric ratio had not altered significantly (33:67), indicating that an equilibrium composition remarkably similar to that observed in chloroform solution, had been rapidly reached. To investigate the possibility that the high salt content of the buffer solution may have induced heating of the sample during radio frequency irradiation, the kinetic test was repeated using a buffer mixture containing no sodium or potassium chloride, but the same result was observed. Since the presence of 16% acetonitrile in the preliminary isomerisation trial did not cause a dramatic increase in isomerisation rate, it appeared that the buffer mixture itself had caused the observed rate increase.



It was thus reluctantly concluded that it was likely that hemiketal A-factor **5.16a** would equilibrate too rapidly during the proposed protein-binding assay for any significant difference in binding capability to be measured. Future approaches to the determination of the biologically active isomer of A-factor may be directed towards:

- development of new methods for bioassay which do not promote isomerisation,
- isolation of the purified binding protein and its crystallisation in its ligand-bound form, (to this end the purification and cloning of the closely related 6-hydroxybutanolide autoregulator binding protein of *S. virginiae* was recently reported<sup>175</sup>),
- preparation of non-isomerisable analogues such as **5.17** ( $X = \text{CH}_2$ ) and the testing of these for possible biological activity.



**5.17**

## 5.6 CONCLUDING COMMENTS

Reassessment of the spectral data of A-factor has led to the discovery of novel bicyclic hemiketal forms of A-factor **5.12** which are in solution equilibrium with the conventionally drawn form of A-factor **1.1a** and which represent over one third of the compound in solution or up to 100% in the absence of solvent. The previous uncertainty over the isomeric structure of this potent autoregulator has now been resolved, allowing definitive assignment of NMR data. The unique crystalline form of A-factor, the 6-*endo*-hydroxy hemiketal **5.12a**, in addition to facilitating X-ray crystallographic determination of relative stereochemistry also provides a convenient and stable form in which to store the autoregulator. The hemiketal isomers of A-factor



have been shown to react readily with alcohols to form ketals, a fact which will be of considerable interest to the commercial supplier of A-factor, Funakoshi Co. Ltd., who currently follow the potentially risky practice of supplying A-factor as an ethanol solution. Supply of the product in the stable crystalline 6-*endo*-hydroxy hemiketal form **5.12a** may circumvent the current requirement for expensive and inconvenient dry-ice cooling during transportation (probably only required to minimise reaction with ethanol). It has also been demonstrated that our synthetic route to A-factor provides an improved method for the introduction of a non-exchanging label.

## CHAPTER SIX

### THE 6-HYDROXYBUTANOLIDE AUTOREGULATORS:

### SYNTHESIS AND STRUCTURAL REVISION

*If error is corrected whenever it is recognised as such,  
then the path of error is the path of truth.*

Hans Reichenbach (1892-1953)



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## 6.1 INTRODUCTION

Following the isolation of A-factor (1.1) from cultures of *S. griseus*,<sup>15</sup> an expanding series of related butanolide autoregulators incorporating a 6-hydroxy rather than a 6-keto group has been isolated from various *Streptomyces* species (Table 6.1, 1.2-1.10). The occurrence and biological activities of these compounds were discussed in Chapter 1.

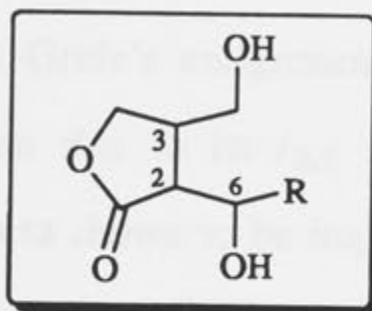
## 6.2 REPORTED STEREOCHEMICAL ASSIGNMENTS

### 6.2.1 2,3-Relative Stereochemistry

The first 6-hydroxybutanolide isolated, by Gräfe and co-workers in 1982, was factor-I (1.2) from *S. viridochromogenes*.<sup>26</sup> As mentioned earlier in this thesis, its stereochemistry was assigned as 2,3-*trans* on the basis that its  $J_{2,3}$  coupling constant (9.4 Hz) lies within the range earlier reported by Savostianoff and Pfau<sup>157</sup> for a variety of 2,3-*trans* substituted  $\gamma$ -lactones (9-12 Hz) despite the fact that the 2,3-substituents in factor-I were quite different. This assignment appeared to be supported soon after by the isolation<sup>28</sup> from *S. bikiniensis* and *S. cyaneofuscatus* of three further 6-hydroxybutanolide autoregulators 1.4, 1.5 and 1.6, of which 1.5 was diastereomeric with factor-I (1.2). All of these later isolated compounds displayed a common vicinal  $J_{2,3}$  value of 7.26 Hz which was in the centre of the range observed by Savostianoff and Pfau<sup>157</sup> for 2,3-*cis* substituted  $\gamma$ -lactones (5-9 Hz).



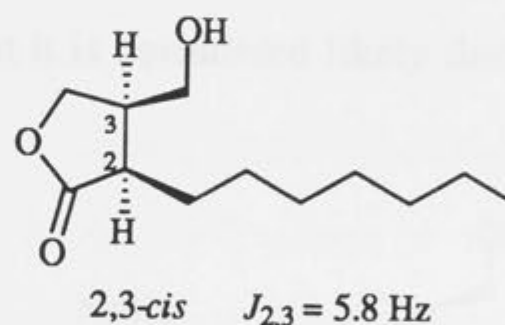
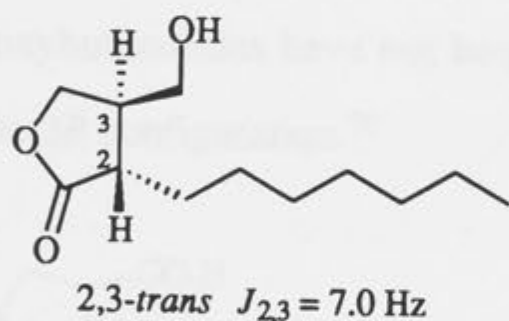
Table 6.1 Reported Structures of 6-Hydroxybutanolide Autoregulators  
Isolated from *Streptomyces*



2,3-Relative Stereochemical Assignment (Literature)	Auto- regulator	Alkyl substituent 'R'	<i>Streptomyces</i> Species	$J_{2,3}$ (Hz) CDCl <sub>3</sub>	$J_{2,6}$ (Hz) CDCl <sub>3</sub>	Reference
2,3- <i>trans</i>	factor-I 1.2		<i>viridochromogenes</i>	9.4	4.6	26
"	IM-2 1.3		<i>FRI-5</i>	9.3 (CDCl <sub>3</sub> ) 6.1 (D <sub>2</sub> O)	- 3.46 (D <sub>2</sub> O)	36
2,3- <i>cis</i>	1.4		<i>bikiniensis</i> and <i>cyaneofuscatus</i>	7.26	3.81	28
"	1.5		<i>bikiniensis</i> and <i>cyaneofuscatus</i>	7.26	3.81	28
"	VB-A 1.6		<i>cyaneofuscatus</i> and <i>virginiae</i>	7.26 7.40	3.81 3.77	28 25
"	VB-B 1.7		<i>virginiae</i>	7.26	3.63	25
"	VB-C 1.8		<i>virginiae</i>	7.26	3.63	25
"	VB-D 1.9		<i>virginiae</i>	7.3	3.7	24
"	VB-E 1.10		<i>virginiae</i>	7.5	3.6	24

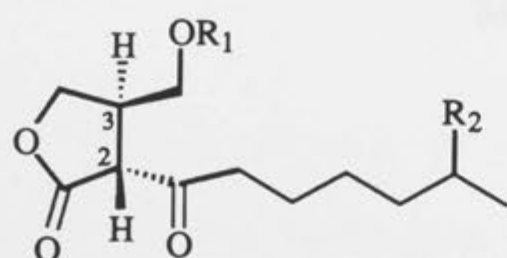
When Yamada's group subsequently isolated the 'virginiae butanolides' (VB-A 1.6, VB-B 1.7, VB-C 1.8, VB-D 1.9 and VB-E 1.10),<sup>24,25</sup> they were also found to display  $J_{2,3}$  coupling constants of about 7.3 Hz, thus all were assigned 2,3-*cis* configuration by analogy with Gräfe's assignments. Similarly IM-2 (1.3)<sup>36</sup> was assigned 2,3-*trans* configuration due to its  $J_{2,3}$  value of 9.3 Hz. The relative stereochemistry of C2 and C3 was shown to be important for the antibiotic inducing activity of the virginiae butanolides (1.6-1.10). A structure-activity relationship study by Yamada's group on forty one synthetic analogues incorporating a variety of structural modifications showed that, for a given alkyl chain length, those assigned as '2,3-*cis*' were ten times more active in inducing antibiotic production in *S. virginiae* than the '2,3-*trans*' type.<sup>35</sup>

Interestingly, one '2,3-*trans*' compound used in this study lacking oxygen functionality at C6 used in this study displayed a  $J_{2,3}$  value of only 7.0 Hz which is significantly below the range of 9-12 Hz observed by Savostianoff and Pfau, whilst its '2,3-*cis*' diastereomer also showed the relatively low  $J_{2,3}$  value of 5.8 Hz.<sup>157</sup>



In combination with our own observation of low  $J_{2,3}$  values for 2,3-*trans*  $\beta$ -keto- $\gamma$ -lactones (Table 6.2), this shows that significant overlap exists between the possible *cis* and *trans*  $J_{2,3}$  ranges. Thus the empirical correlation between  $J$  value and relative configuration is risky, especially when all diastereomers of a given compound are not available for comparison.

Table 6.2  $J_{2,3}$  Couplings in A-Factor and Derivatives



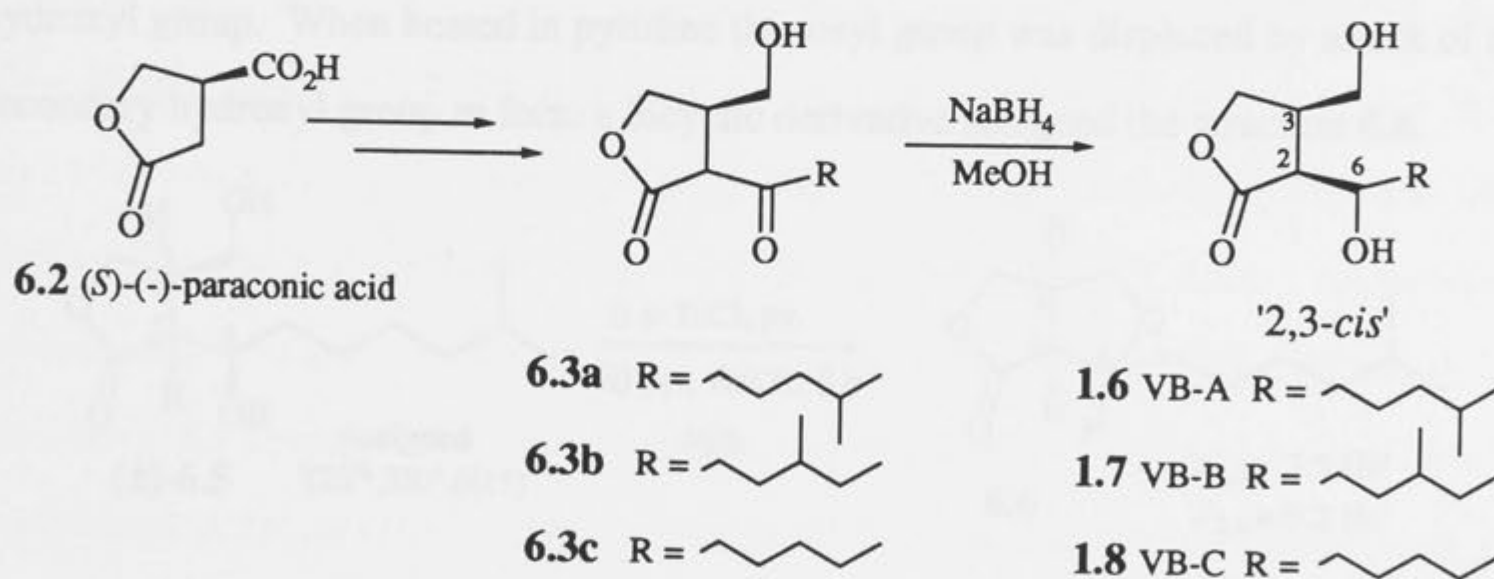
	$R_1$	$R_2$	$J_{2,3}$ (Hz)
1.1	H	Me	7.0
4.35	TBDMS	Me	6.8
4.36	H	H	7.0
4.27	TBDMS	H	6.8



### 6.2.2 Absolute Configuration at C3

At the start of the present study no definite proposals had been made regarding the absolute configuration of the 6-hydroxybutanolide autoregulators. Since it appeared likely that these compounds are produced biosynthetically *via* reduction of a 6-keto A-factor-type intermediate, we thought it plausible that they would share the 3*R* configuration of A-factor (1.1).

This was recently confirmed by Mori and Chiba<sup>30</sup> who synthesised the virginiae butanolides A-C (1.6-1.8) *via* sodium borohydride reduction of optically pure (3*R*)-A-factor analogues (6.3a-c, Scheme 6.1), which were themselves prepared from (*S*)-(-)-paraconic acid (6.2) using the same strategy as in their asymmetric synthesis of A-factor<sup>17</sup> (1.1) (see Section 1.6.1 Scheme 1.4). The minor products from these reductions of A-factor analogues (6.3a-c), assigned as '2,3-cis', were identified as the natural virginiae butanolides A, B and C (1.6, 1.7 and 1.8) by the correlation of high field NMR data and CD spectra.<sup>24,25</sup> Chiroptical data for the other naturally occurring 6-hydroxybutanolides have not been reported but it is considered likely that they too share the 3*R* configuration.<sup>30</sup>



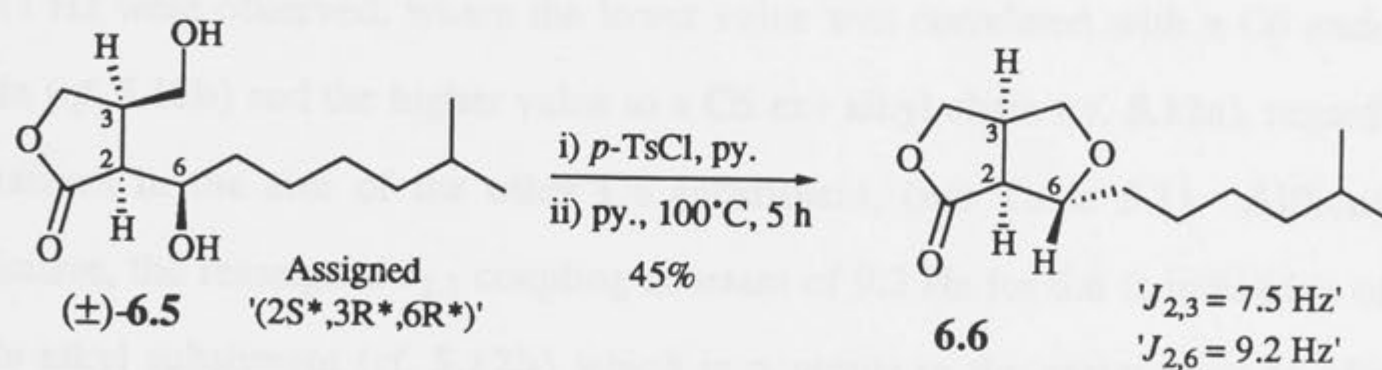
Scheme 6.1 Asymmetric Synthesis of Virginiae Butanolides A-C by Mori and Chiba



### 6.2.3 Configuration of the 6-Hydroxy Substituent

All of the '2,3-*cis*' 6-hydroxybutanolide autoregulators so far discovered (Table 6.1, 1.4-1.10) display very similar  $J_{2,6}$  values (3.6-3.81 Hz), which has led to the not unreasonable proposal that their configurations at C6 are probably the same.<sup>25</sup> Following the success of their assignment of the absolute configuration at C3 of the virginiae butanolides, Mori and Chiba then attempted to assign the absolute configuration of the 6-hydroxy substituents.<sup>30</sup> Two independent methods were used but unfortunately both appear to be seriously flawed.

Their first method involved an attempt to establish the C2-C6 relative configuration of the '2,3-*cis*' autoregulator ( $\pm$ )-**6.5** by stereochemical analysis of its rigid 2,3-*cis*-bicyclic derivative **6.6** (Scheme 6.2). The autoregulator ( $\pm$ )-**6.5** was obtained as the minor diastereomer from the sodium borohydride reduction of ( $\pm$ )-A-factor (**1.1**) and was identical by NMR to Gräfe's factor **1.5**. The 2,6-configuration of ( $\pm$ )-**6.5** was assumed to be common to all '2,3-*cis*' 6-hydroxybutanolide autoregulators because of the close correspondence of vicinal coupling values (1.4-1.10, Table 6.1). Treatment of ( $\pm$ )-**6.5** with *p*-toluenesulfonyl chloride in pyridine caused tosylation of the primary hydroxyl group. When heated in pyridine the tosyl group was displaced by attack of the secondary hydroxyl group to form a bicyclic derivative assigned the structure **6.6**.



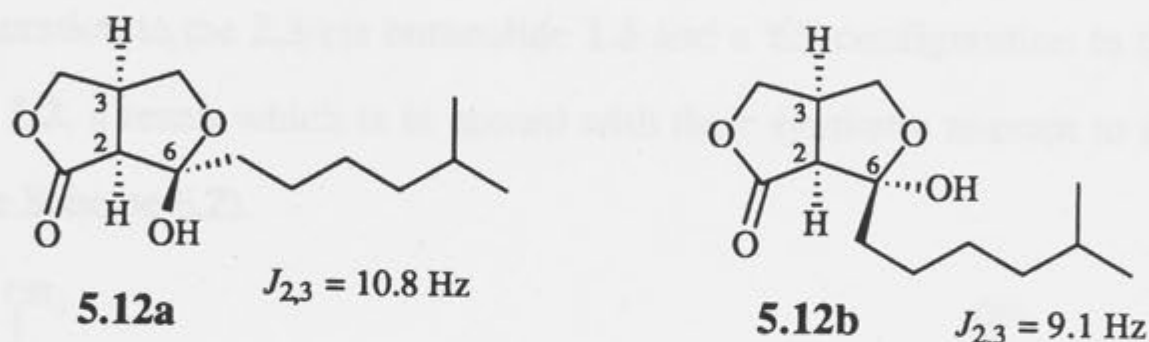
**Scheme 6.2** Attempt by Mori and Chiba to Define the C6 Relative Configuration of a '2,3-*cis*' Butanolide

The  $^1\text{H}$  NMR spectrum of **6.6** displayed the H2 proton as a doublet of doublets ( $\delta$  3.10) with coupling constants of 7.5 and 9.2 Hz. These coupling constants were assigned as  $J_{2,3}$  and  $J_{2,6}$  respectively, in the absence of confirmatory vicinal coupling



values from the H3 or H6 multiplets. Although not stated, the lower coupling constant was probably assigned to accord with the 7.3-7.5 Hz range for the  $J_{2,3}$  values of 'cis' compounds as shown in Table 6.1. Mori and Chiba then asserted that the value of 9.2 Hz was "in accord with ... 2,6-anti configuration" (i.e. *exo*-alkyl chain, **6.6**) and that since the cyclisation was achieved with retention of configuration at C6, then the starting butanolide (and hence **1.5**) must have had the indicated  $3R^*$ ,  $6R^*$  configuration (**6.5**, Scheme 6.2).

However, the A-factor bicyclic hemiketals (**5.12a** and **5.12b**) which we have isolated (see Chapter 5) represent very close NMR models for the bicyclic  $\gamma$ -lactone **6.6** and display  $J_{2,3}$  values of 10.8 and 9.1 Hz respectively.



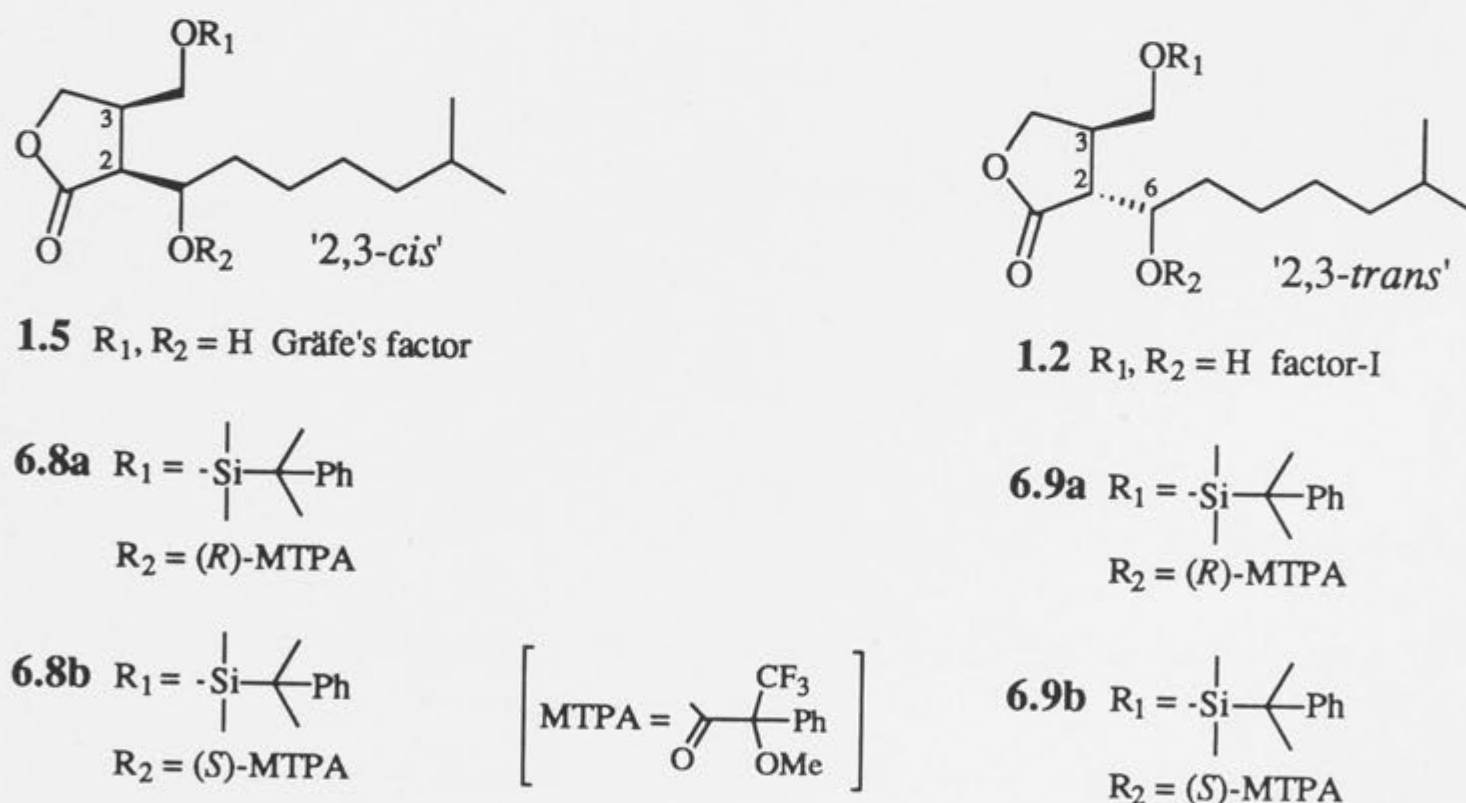
Thus it is highly likely that Mori and Chiba incorrectly assigned the  $J_{2,3}$  and  $J_{2,6}$  couplings, casting doubt upon their stereochemical conclusions. Furthermore, in Chapter 5 (Section 5.4.3, Table 5.2) we showed that for the diastereomeric pairs of *cis*-bicyclic  $\gamma$ -lactones (**5.12**, **5.9** and **5.8**),  $J_{2,3}$  coupling constants of either *ca* 9 Hz or *ca* 11 Hz were observed, where the lower value was correlated with a C6 *endo* alkyl chain (cf. **5.12b**) and the higher value to a C6 *exo* alkyl chain (cf. **5.12a**), regardless of variations in the size of the other C6 substituent, (see Table 5.1). Although not definitive, the reassigned  $J_{2,3}$  coupling constant of 9.2 Hz for **6.6** is indicative of a C6 *endo* alkyl substituent (cf. **5.12b**) which is contrary to the assignment of Mori and Chiba.

In an attempt to confirm their C6 assignment, Mori and Chiba utilised a high-field  $^1\text{H}$  NMR version of Mosher's method<sup>176</sup> to predict the absolute configurations at the secondary alcohol position of the optically active butanolide autoregulators ( $3R$ )-**1.5** and ( $3R$ )-**1.2** obtained by reduction of ( $3R$ )-A-factor (**1.1**).<sup>30</sup>



This version of Mosher's method, first described by Tanako *et al.*<sup>177</sup> involves the preparation of both (*R*)- and (*S*)- $\alpha$ -methoxy- $\alpha$ -(trifluoromethyl)phenylacetates (MTPA) of a secondary alcohol and comparison of the  $^1\text{H}$  chemical shift changes induced in the other proton resonances of the molecules by the phenyl groups of the chiral acyl moieties. The direction of the  $^1\text{H}$  chemical shift differences ( $\Delta\delta$ ) observed between protons of the two diastereomeric MTPA esters can then be empirically correlated with a particular absolute configuration of the alcohol.<sup>176-178</sup>

Accordingly, Mori and Chiba converted the (3*R*)-6-hydroxybutanolides **1.5** and **1.2** into two pairs of silyl protected (*R*)- and (*S*)-MTPA esters (**6.8a**, **6.8b** and **6.9a**, **6.9b** respectively). They used the 500 MHz  $^1\text{H}$  NMR data of these derivatives to assign a '*6R*' configuration to the 2,3-*cis* butanolide **1.5** and a '*6S*' configuration to the 2,3-*trans* butanolide **1.2**, a result which is in accord with their synthetic attempt to assign C6 in ( $\pm$ )-**6.5** (see Scheme 6.2).



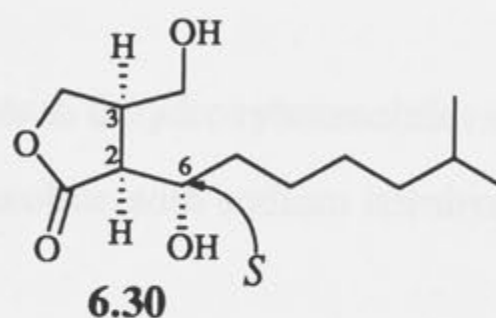
**Scheme 6.3** Substrates Prepared by Mori and Chiba for Determination of C6 Absolute Configuration by Mosher's Method

However, we have re-examined these data using both the original description of the method<sup>176</sup> and the recent lucid explication of the required analysis given by Ohtani *et al.*<sup>178</sup>. This revealed firstly that the reported data are deficient in key areas, and secondly that when analysed properly, they yield the opposite stereochemical

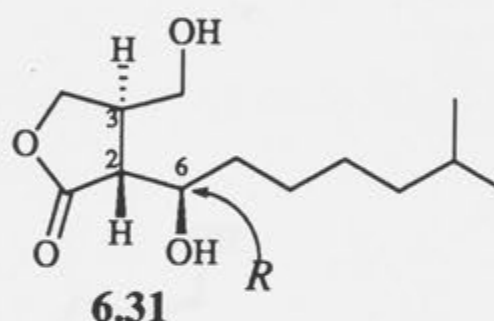


assignments to those reported, *i.e.* (6*S*)-**1.5** (structure **6.30**) and (6*R*)-**1.2** (structure **6.31**) [see Appendix F for the revised data analysis]. The reassignment of **1.5** would support our tentative reassignment of the 2,6-relative configuration of the bicyclic derivative of (±)-**6.5** (Scheme 6.2). Structures **6.30** and **6.31** below represent our tentative C6 stereochemical reassignment of autoregulators **1.5** and **1.2**.

Tentative Stereochemical  
Reassignment of **1.5**



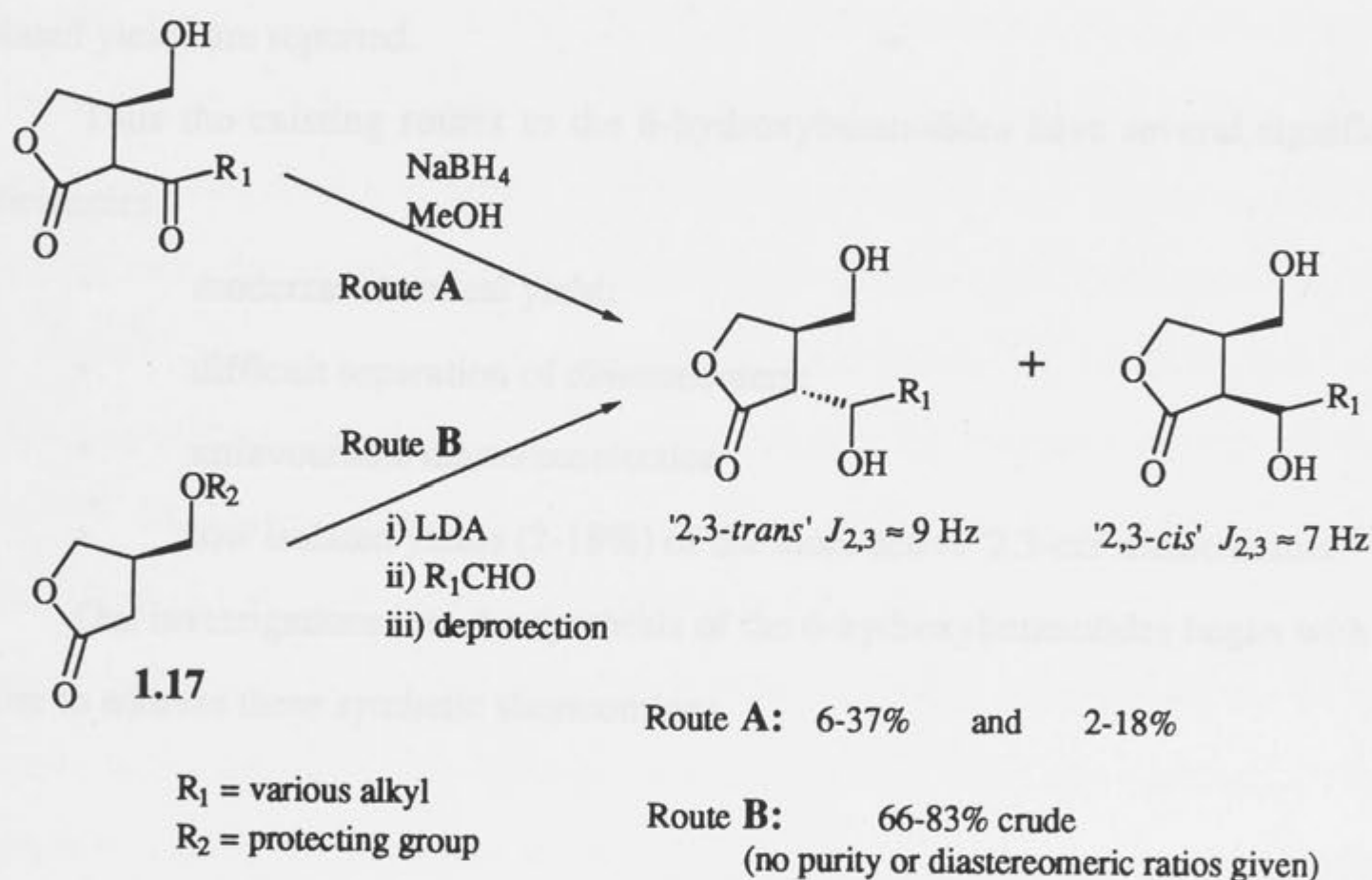
Tentative Stereochemical  
Reassignment of **1.2**



### 6.3 SYNTHESIS OF THE 6-HYDROXYBUTANOLIDE AUTOREGULATORS

#### 6.3.1 Existing Routes

The most commonly employed route to 6-hydroxybutanolides is reduction of the corresponding 6-keto (A-factor type) butanolide with sodium borohydride in methanol (Scheme 6.4, Route A).<sup>25,30,35</sup>



**Scheme 6.4 Existing Routes to 6-Hydroxybutanolide Autoregulators**

A wide range of 6-hydroxybutanolides has been prepared by this method, including a racemic series containing *n*-alkyl substituents ( $\text{R}_1 = \text{CH}_3 - \text{C}_9\text{H}_{19}$ )<sup>25,35</sup> and the pair of diastereomeric autoregulators factor-I (1.2) and Gräfe's factor (1.5) as well as the optically active natural virginiae butanolides A-D (1.6-1.9).<sup>30</sup> The reduction gives two diastereomeric products in a 2:1 to 3:1 ratio (isolated) which have been assigned '2,3-trans' and '2,3-cis' relative stereochemistry respectively, on the basis of their  $J_{2,3}$  coupling constants (as discussed above). In the only case where the diastereomeric ratio present in the crude reaction mixture was measured ( $\text{R} = n\text{-C}_5\text{H}_{11}$ ), a ratio of 2.5:1 was



reported.<sup>25</sup> Isolated yields of the products are moderate to low due to both the chemical yield and to the difficulty in chromatographically separating the diastereomers. Even using reverse-phase preparative HPLC, separation is only partial and yields of purified diastereomers are low.<sup>25,35</sup>

A related mixture of diastereomers, also favouring the '2,3-*trans*' form, is isolated from the alternative synthetic route (Route B, Scheme 6.4) in which the anion of the protected 3-hydroxymethylbutanolide **1.17** is alkylated with an aldehyde. However, for the only two examples reported [ $R_1 = (\text{CH}_2)_4\text{CH}(\text{CH}_3)_2$ ,  $R_2 = \text{SiMe}_3$ <sup>47</sup> and  $R_1 = (\text{CH}_2)_4\text{CH}(\text{CH}_3)_2$ ,  $R_2 = \text{CH}_2\text{Ph}$ <sup>58</sup>] no quantification of diastereomeric ratios or isolated yields are reported.

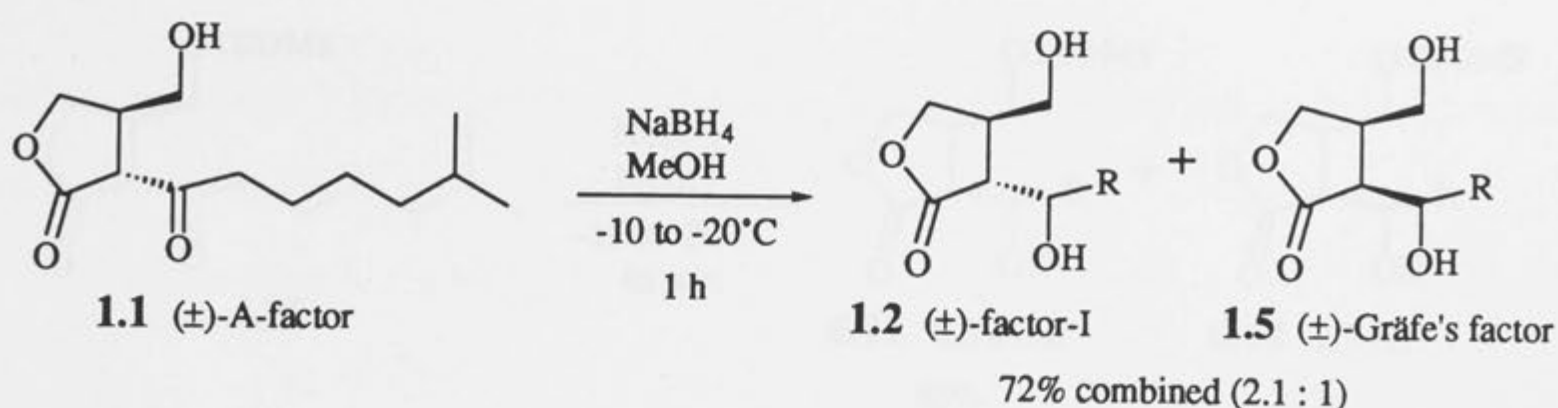
Thus the existing routes to the 6-hydroxybutanolides have several significant deficiencies :

- moderate chemical yield;
- difficult separation of diastereomers;
- unfavourable diastereoselection
- low isolated yields (2-18%) of the more active '2,3-*cis*' diastereomer.<sup>35</sup>

Our investigations into the synthesis of the 6-hydroxybutanolides began with the desire to address these synthetic shortcomings.

### 6.3.2 Improved Synthesis of 6-Hydroxybutanolides *via* the Reduction of 6-Ketobutanolides

To obtain comparative data on the reported reduction of (±)-A-factor (**1.1**) (Method A, Scheme 6.4) the reaction was repeated essentially as described by Mori and Chiba,<sup>30</sup> except that a lower reaction temperature was employed (Scheme 6.5).



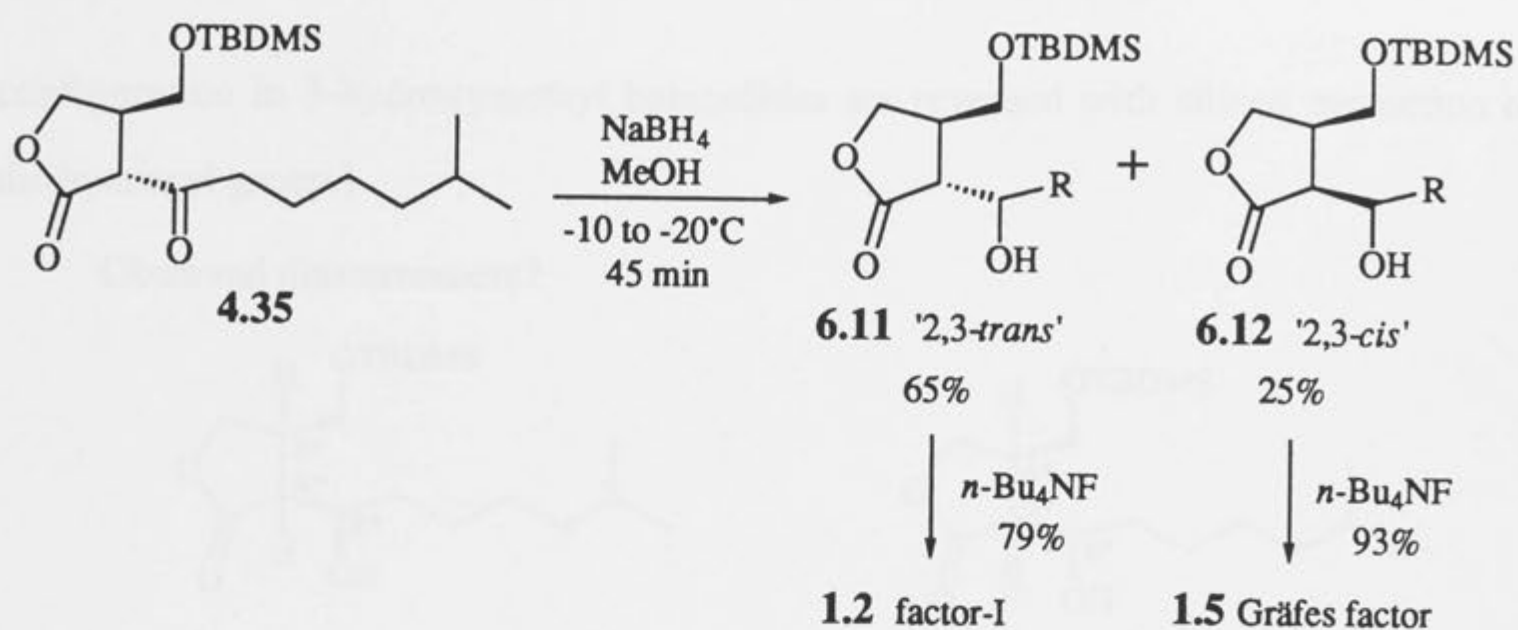
[cf Mori and Chiba  $0$ – $20^\circ\text{C}$ , 1 h giving 37% : 18% isolated; 2.1 : 1]

### Scheme 6.5 Reduction of A-Factor with Sodium Borohydride

$^1\text{H}$  NMR analysis of the crude reaction mixture showed, as reported by Mori and Chiba, two products in a 2:1:1 ratio which corresponded well with the 200 MHz  $^1\text{H}$  NMR data reported for factor-I (1.2)<sup>26</sup> and Gräfe's factor from *S. bikiniensis* and *S. cyaneofuscatus* (1.5).<sup>28</sup> Radial chromatography in several solvent systems failed to separate the diastereomers, which appeared as a single spot by TLC, and they were collected as a mixture in 72% yield. The chromatographic separation reported by Mori and Chiba<sup>30</sup> failed to give adequate separation in our hands.  $^{13}\text{C}$  NMR analysis of the mixture confirmed the identity of the diastereomers by comparison with published data.<sup>26,28</sup> The mixture clearly consisted of only two diastereomers and no evidence of other 6-hydroxy diastereomers was observed in any other fraction. Small amounts of several unidentified polar byproducts were collected in late fractions but by  $^1\text{H}$  NMR spectroscopy these were very different in structure from the major products. Thus it appears that using this reduction, the desirable '2,3-*cis*' diastereomer would be available in a maximum of *ca* 23% yield (cf. 18% by Mori and Chiba<sup>30</sup>) even if it were possible to efficiently separate the diastereomers.

In an attempt to improve the yield and possibly alter the product ratio, the reduction was repeated using TBDMS-protected (±)-A-factor (4.35), a stable and easily purified intermediate from our synthesis of A-factor.





**Scheme 6.6** Reduction of TBDMS Protected A-Factor

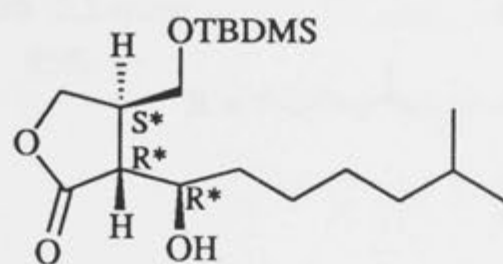
The resulting reaction mixture again showed two diastereomers (**6.11** and **6.12**) with  $^1\text{H}$  NMR resonances directly analogous to the unprotected products **1.2** and **1.5**. Unfortunately, the proportion of the desired '2,3-cis' diastereomer **6.12** was marginally lower (**6.11** : **6.12**, 2.7 : 1) but, in compensation, the two products could be much more easily resolved by chromatography ( $R_f$  0.73 vs 0.52, 4% MeOH in  $\text{CH}_2\text{Cl}_2$ ) and the combined yield of diastereomers was significantly increased (90%). Radial chromatography afforded the pure diastereomers in 65% and 25% yields respectively. The separated diastereomers were then deprotected using *tetra-n*-butylammonium fluoride to give ( $\pm$ )-factor-I (**1.2**) and ( $\pm$ )-Gräfe's factor (**1.5**) in 79% and 93% yields respectively, establishing a more convenient and higher yielding route to these compounds.

In an attempt to rationalise the unfavourable diastereomeric ratio formed on reduction of the 6-keto compounds **4.35** and **1.1** we considered the stereochemical course of the reaction more closely. Firstly, it was not at all clear why the reductions should give only two diastereomeric products when four are theoretically possible, two 2,3-cis and two 2,3-trans products. Assuming that our tentative reassignment of the C6 stereochemistry of the reduction products **1.2** and **1.5** obtained by Mori and Chiba<sup>30</sup> was correct (see Section 6.2.3, structures **6.30** and **6.31**) then it appeared likely that the products we observed from the reduction of TBDMS protected A-factor (**4.35**) (*i.e.* **6.11** and **6.12**) should have the relative stereochemistry  $2R^*$ ,  $3S^*$ ,  $6R^*$ , and  $2S^*$ ,  $3S^*$ ,  $6S^*$  respectively, as shown in Scheme 6.7. [Note that the stereochemical designations of C3

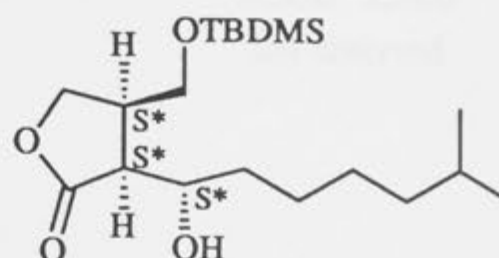


configuration in 3-hydroxymethyl butanolides are reversed with silicon protection of the hydroxyl group.]

Observed diastereomers?

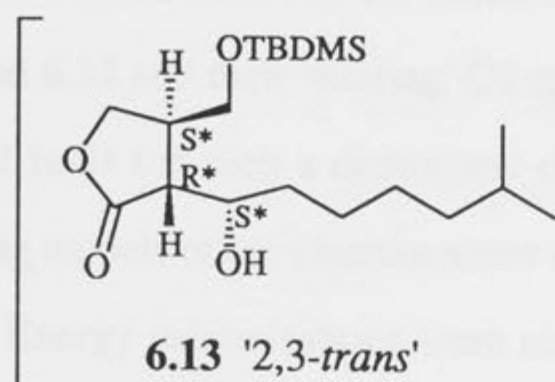


6.11 '2,3-*trans*'

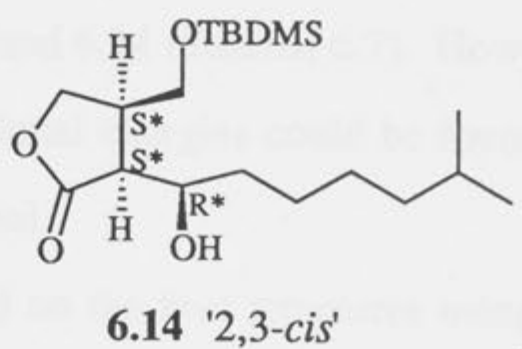


6.12 '2,3-*cis*'

'Missing' diastereomers?



6.13 '2,3-*trans*'

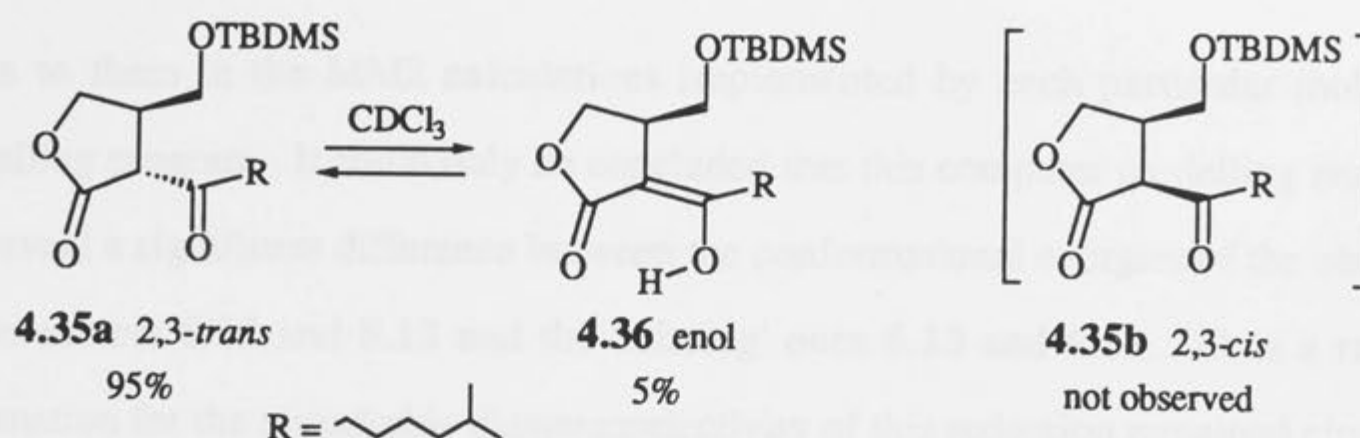


6.14 '2,3-*cis*'

Scheme 6.7

Thus the generation of the two alternative diastereomers **6.13** and **6.14**, which are C6 epimers of the observed products, appeared to be inhibited in some way. Other groups who have performed directly analogous reactions seem unsurprised by this exceptional diastereoselectivity, since none have commented on it.<sup>25,30,35</sup> Although not stated, Yamada implies in his schemes that the '2,3-*trans*' : '2,3-*cis*' product ratio follows from the diastereomeric ratio of the 6-keto starting material, in which the major form was also presumed to have '2,3-*trans*' geometry. However, we have shown in Chapter 5 that 6-ketobutanolides [*e.g.* A-factor (**1.1**), see Scheme 5.4a] do not exist to any significant extent in the 2,3-*cis* 6-keto form **1.1c** but rather as the 2,3-*cis* bicyclic hemiketals **5.12a** and **5.12b**. Furthermore, the TBDMS-derivative **4.35** has been shown to exist in solution only in the 2,3-*trans* keto **4.35a** and enol **4.36** forms (95:5 mixture, CDCl<sub>3</sub>) (Scheme 6.8). No evidence exists for any contribution of the 2,3-*cis* keto form **4.35b**, and therefore it is unlikely that the '2,3-*cis*' reduction product derives from this tautomer simply *via* a face selective reduction of its ketone carbonyl.





Scheme 6.8

The possibility that the composition of the mixture of reduction products is thermodynamically controlled *via* C2 epimerisation would require that a substantial difference exists between the conformational energies of the observed diastereomers **6.11** and **6.12** and their 'missing' C6 epimers, **6.13** and **6.14** (Scheme 6.7). However no rational basis for such a dichotomy of conformational energies could be found when Dreiding models of the diastereomers were compared.

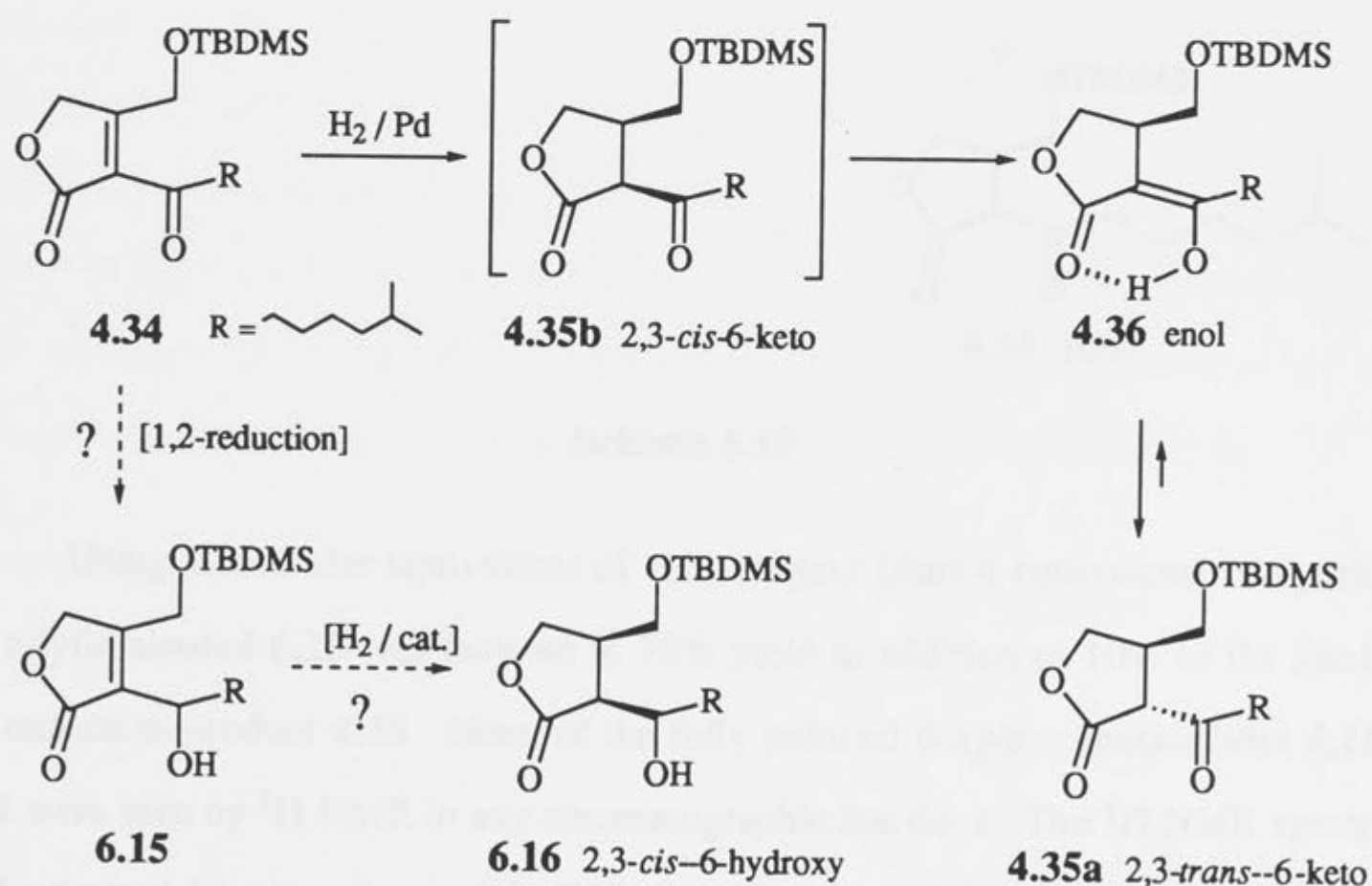
Energy minimisations were also conducted on the four structures using MM2 force-field calculations as implemented in PC Model (version 88.0). Special attention was given to the possibility of forming intramolecular H-bonds between the 6-hydroxyl and the lactone carbonyl since it was felt that this feature could potentially confer significant stabilisation to any diastereomers in which it was present. Results obtained after many minimisations of each diastereomer (starting from a variety of conformations) were that the minimum energy conformations were all within approximately 3 kcal/mol of each other, with the '2,3-*trans*' diastereomers **6.11** and **6.13** being generally slightly lower in energy (*ca* 1-2 kcal/mol) than the '2,3-*cis*' forms **6.12** and **6.14**, as expected on the basis of steric hindrance. Interestingly, none of the lowest energy conformations displayed the postulated H-bond, even when the structures to be minimised were started with the H-bond in place. It was recognised that considerable caution must be used in the interpretation of results from iterative MM2 energy minimisation studies of this sort, since all conformations of all bonds have not been searched, and consequently the low energy conformations revealed may only represent local minima. In addition, H-bonding effects depend significantly on the emphasis



given to them in the MM2 calculations implemented by each particular molecular modelling program. It could only be concluded that this computer modelling study did not reveal a significant difference between the conformational energies of the observed diastereomers **6.11** and **6.12** and the 'missing' ones **6.13** and **6.14**. Thus a rational explanation for the remarkable diastereoselectivity of this reduction remained elusive at this stage.

### 6.3.3 Synthesis of 2,3-*cis* 6-Hydroxybutanolides via Catalytic Hydrogenation of 6-Hydroxybutenolides

Since reduction of 6-ketobutanolides formed a discouragingly low proportion of the more active '2,3-*cis*' 6-hydroxy diastereomer, a more direct synthetic approach to their synthesis was sought.



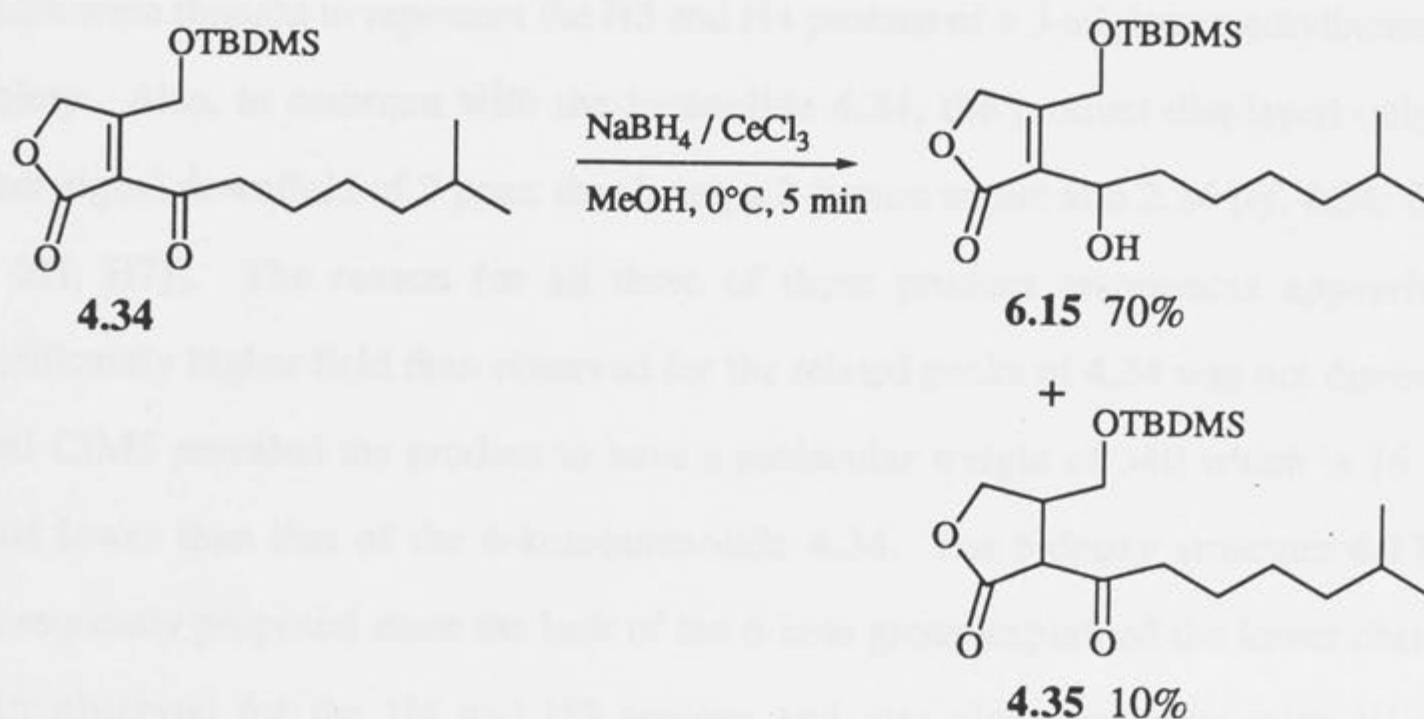
Scheme 6.9

In Chapter 4 (Section 4.2.3) it was shown that catalytic hydrogenation of the butenolide intermediate **4.34** gave the 2,3-*trans* product **4.35a** even though the hydrogen atoms were presumably delivered *cis* from the catalyst surface, initially forming the 2,3-*cis* diastereomer **4.35b**. Conversion to the more thermodynamically



stable 2,3-*trans* diastereomer could readily occur even under essentially neutral conditions *via* the enol tautomer **4.36** due to the considerable acidity of the H2 proton and to the stability gained in the enol form by intramolecular H-bonding. It was reasoned that if the acidity of the hydrogenation product could be diminished by prior reduction of the activating ketone (*i.e.* **6.15**) then it may be possible to retain the initially-formed 2,3-*cis* configuration (**6.16**, Scheme 6.9).

Thus the 2-acyl butenolide **4.34** was reduced using sodium borohydride in methanol in the presence of anhydrous cerium trichloride, conditions which promote selective 1,2-reduction<sup>179</sup> (Scheme 6.10).



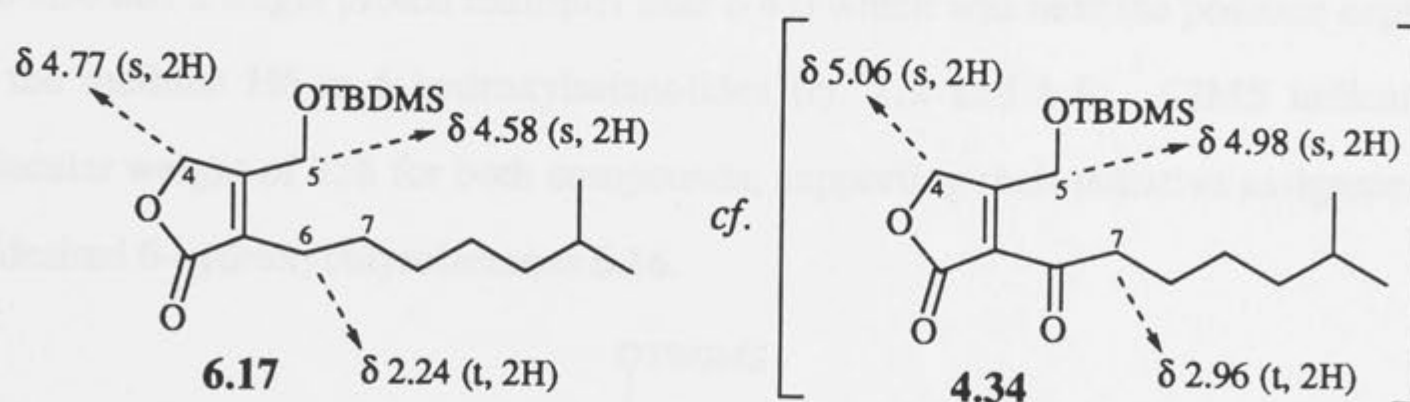
Scheme 6.10

Using 1.05 molar equivalents of each reagent (thus 4 equivalents of hydride), the allylic alcohol **6.15** was isolated in 70% yield in addition to 10% of the familiar 1,4 reduction product **4.35**. None of the fully reduced 6-hydroxybutanolides **6.11** or **6.12** were seen by <sup>1</sup>H NMR in any chromatographic fractions. The <sup>1</sup>H NMR spectrum of the desired 6-hydroxybutenolide **6.15** showed the new C6 proton as a pseudo triplet at  $\delta$  4.51 and the hydroxyl proton as a broad resonance at  $\delta$  2.70. In addition, the creation of the new C6 chiral centre caused a chemical shift difference in the H5a and H5b protons such that they appeared as two narrowly separated doublets at  $\delta$  4.73 and 4.66, rather than as the 2 proton singlet observed with the starting butenolide **4.34**.



Catalytic hydrogenation of the allylic alcohol **6.15** proved to be more complex than initially envisaged. The substrate was hydrogenated over 5% palladium-on-carbon in methanol until 1 equivalent of hydrogen had been absorbed (3 h) forming a mixture of four products in addition to a small amount of unreacted starting material. After chromatographic separation of these products, we were surprised to find that by  $^1\text{H}$  NMR spectroscopy *none* of them corresponded to any previously isolated products.

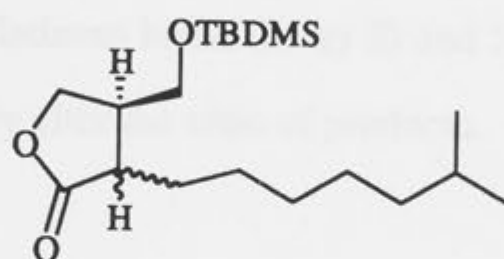
The  $^1\text{H}$  NMR spectrum of the major product (47% yield) was very simple and appeared to resemble that of the 6-ketobutenolide **4.34**. It possessed two 2-proton singlets at  $\delta$  4.58 and 4.77 [*cf.* butenolide **4.34**:  $\delta$  4.98 (s, 2H, H5), 5.05 (s, 2H, H4)] which were thought to represent the H5 and H4 protons of a 3-silyloxymethylbutenolide moiety. Also, in common with the butenolide **4.34**, the product displayed only one other signal downfield of 2 ppm; that being a 2-proton triplet at  $\delta$  2.24 [*cf.* **4.34**:  $\delta$  2.96 (t, 2H, H7)]. The reason for all three of these product resonances appearing at significantly higher field than observed for the related peaks of **4.34** was not discovered until CIMS revealed the product to have a molecular weight of 340 which is 16 mass units lower than that of the 6-ketobutenolide **4.34**. The 6-deoxy structure **6.17** was subsequently proposed since the lack of the 6-keto group explained the lower chemical shift observed for the H4 and H5 protons and was also consistent with all other spectroscopic data for the compound including  $^{13}\text{C}$  NMR, EIMS and HRMS.



The 6-deoxy product **6.17** is apparently formed by catalytic hydrogenolysis of the allylic hydroxyl group of **6.15**, a process which reportedly often competes with catalytic hydrogenation when substrates contain an allylic carbon-oxygen linkage, particularly when the double bond is tetrasubstituted.<sup>154</sup>

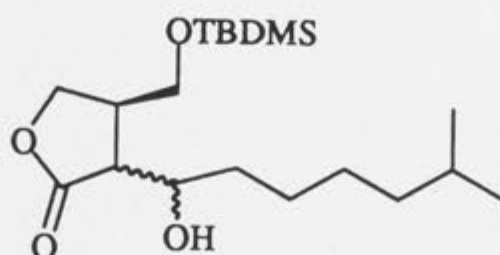


In contrast with the major product **6.17**, the second most abundant hydrogenation product (17%) displayed a highly coupled  $^1\text{H}$  NMR spectrum. Peak integration suggested the presence of two closely related compounds in a 70:30 ratio. Their CIMS indicated that they shared a molecular weight of 342, which is two mass units higher than the 6-deoxy butanolide **6.17**. Assignment of their structures as the saturated 6-deoxy diastereomers **6.18** (presumably derived *via* hydrogenation of the butanolide **6.17**) was supported by  $^{13}\text{C}$  NMR spectroscopy, EIMS and HRMS. The C2-C3 relative geometries of the diastereomers **6.18** could not be readily assigned as their  $J_{2,3}$  values were not discernible from the complex multiplet ( $\delta$  2.38-2.62) which represented their combined H2 and H3 resonances, but it was considered likely that the major product would have 2,3-*cis* configuration resulting from *cis* delivery of hydrogen.



**6.18** 2 diastereomers (70:30)

The third and fourth products (4% each) appeared to be diastereomeric by the marked similarity of their  $^1\text{H}$  NMR spectra. Both displayed the now familiar, highly coupled 6-proton spin system that is characteristic of a saturated butanolide moiety but both also had a single proton multiplet near  $\delta$  4.0 which was near the position expected for the methine H6 in 6-hydroxybutanolides (*cf.* **1.2** and **1.5**). CIMS indicated a molecular weight of 358 for both compounds, supporting their tentative assignment as the desired 6-hydroxybutyrolactones **6.16**.



**6.16** 2 diastereomers

However, we found this assignment difficult to accept since neither compound corresponded to the 6-hydroxy diastereomers **6.11** and **6.12** previously isolated from the

sodium borohydride reduction of TBDMS A-factor **4.35** (see Scheme 6.6). Was it possible that we had unexpectedly isolated the 'missing' diastereomers **6.13** and **6.14**, (Scheme 6.7)? It seemed inconceivable that a single '2,3-*trans*' and a single '2,3-*cis*' diastereomer could be produced by this method. Greater quantities of these intriguing compounds were required for full spectroscopic analysis, so attempts were made to modify hydrogenation conditions to favour hydrogenation over hydrogenolysis.

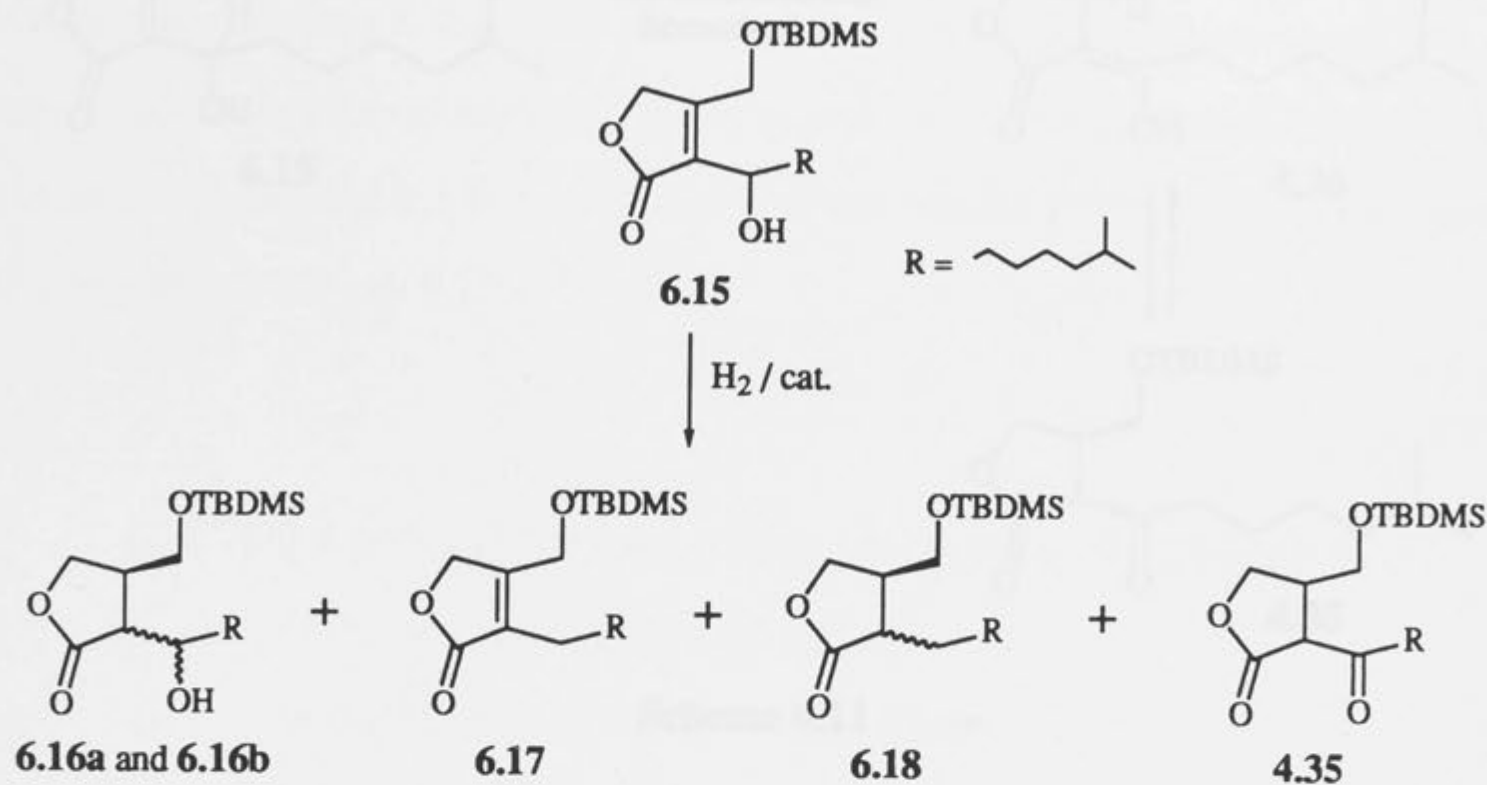
Rylander<sup>154</sup> suggests that choice of catalyst has an influence over the proportion of hydrogenolysis which occurs during hydrogenation of compounds containing allylic oxygen functions. He observes that platinum generally promotes less hydrogenolysis than palladium and rhodium was particularly recommended to prevent the cleavage of allylic hydroxyl groups. The hydrogenation of the 6-hydroxy butenolide **6.15** was repeated (Table 6.3) using platinum black (Entry 2) and 5% rhodium-on-alumina (Entry 3) in an attempt to favourably alter the ratio of products.

1	5% Pd/C	4.00	4.40	32 (40)	38 (77)	5.90	3.40
2	Platinum black	3.00	3.00	5 (4)	12 (10)	2.5 (1.5)	4.40
3	5% Rh/Alumina	3.00	3.00	0 (0)	29 (14)	6.1 (3)	0.90

Hydrogenation with platinum black (Entry 2, Table 6.3) was carried out in benzene since lower solvent polarity has been reported to favour hydrogenation over hydrogenolysis.<sup>155</sup> When using platinum black considerably less of the hydrogenolysed products **6.17** and **6.18** were observed than with the previous palladium on charcoal catalyst (Entry 1, 25% w/w, 1.50%); however a new, compound product was revealed with the identification of 2% of the 4-*trans*-butenolide **4.33** in the reaction mixture. This product presumably results from migration of the double bond (Scheme 6.11) to form **4.34**, the most form of the observed butene **4.35**.



TABLE 6.3 Catalytic Hydrogenation of the 6-Hydroxybutenolide 6.15



Entry No.	Hydrogenation Conditions <sup>iv</sup>	% Product ratio <sup>i</sup> (% Isolated) <sup>ii</sup>					Recovered 6.15
		6.16a	6.16b	6.17	6.18 <sup>iii</sup>	4.35	
1	10% Pd/C MeOH, 3 h	4 (4)	4 (4)	52 (47)	38 (17)	0 (0)	3 (4)
2	Pt black Benzene, 20 h	23 (20)	23 (20)	5 (4)	18 (10)	25 (15)	4 (4)
3	5% Rh/Alumina MeOH, 4.5 h	19 (16)	19 (15)	0 (0)	19 (14)	44 (32)	0 (0) <sup>v</sup>

i) ratio determined by <sup>1</sup>H NMR integration of reaction mixture

ii) isolated percentage yield given in parentheses

iii) 70:30 diastereomeric mixture

iv) all at room temperature and pressure

v) ca 2% each of the other 6-hydroxybutanolides 6.11 and 6.12 were also isolated

Hydrogenation over platinum black (Entry 2, Table 6.3) was carried out in benzene since lower solvent polarity has been reported to favour hydrogenation over hydrogenolysis.<sup>154</sup> When using platinum black considerably less of the hydrogenolysis products 6.17 and 6.18 were observed than with the previous palladium-catalysed reaction (Entry 2, 23% vs Entry 1, 90%). However a new, competing process was revealed with the identification of 25% of the 6-ketobutanolide 4.35 in the reaction mixture. This product presumably results from migration of the double bond (Scheme 6.11) to form 4.36, the enol form of the observed ketone 4.35.





Thus, using platinum black or 5% rhodium-on alumina, moderate yields of the 6-hydroxybutanolides **6.16a** and **6.16b** were obtained. Further investigation into the three competing modes of reaction (*i.e.* hydrogenation / hydrogenolysis / isomerisation) was deferred, pending the full stereochemical elucidation of the 6-hydroxybutanolide diastereomers **6.16a** and **6.16b**.

#### 6.4 RELATIVE STEREOCHEMICAL ASSIGNMENT OF FOUR DIASTEREOMERIC 6-HYDROXYBUTANOLIDES

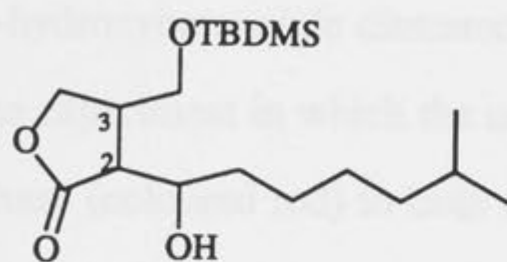
We had thus prepared and purified four different compounds (**6.11**, **6.12**, **6.16a** and **6.16b**) which were shown to be isomeric (CIMS and HRMS) and displayed similar features by  $^1\text{H}$  NMR spectroscopy. Further support for their assignment as diastereomers of the TBDMS-protected 6-hydroxybutanolide was obtained by the very close similarity of their  $^{13}\text{C}$  NMR (APT) spectra. All compounds displayed a C6 methine carbon in the range  $\delta$  70.6-71.1 and all other peaks showed similarly close correspondence (within 3 ppm).

##### 6.4.1 Assignment of 2,3-Relative Stereochemistry

The relative stereochemistry of the C2 and C3 lactone substituents had previously been assigned by other research groups on the basis of the magnitude of the  $J_{2,3}$  coupling constant, as described above. Our misgivings regarding the general applicability of this empirical relationship (see Section 4.2.5) seemed well founded when the two 'new' diastereomers **6.16a** and **6.16b** displayed the  $J_{2,3}$  values of 8.0 and 7.2 Hz respectively (see table below). Not only were these values very close but they are also between the values we measured for the previously isolated diastereomers **6.11** and **6.12** (*i.e.* 8.6 and 7.0 Hz respectively) which had been assigned '2,3-*trans*' and '2,3-*cis*' configurations respectively. Since we were not convinced of the validity of



assigning stereochemistry on the basis of a coupling constant difference of less than 1 Hz, a different and more reliable method was sought.



Four diastereomers

	6.11	6.12	6.16a	6.16b
$J_{2,3}$ (Hz)	8.6	7.2	8.0	7.2

The nuclear Overhauser effect (NOE) is a very useful NMR technique for the elucidation of molecular conformations, since it can provide information on interatomic distances. It is generally performed by the saturation of a particular proton resonance, followed by observation of the effect on the NMR peak intensity of other proton resonances in the molecule caused by through-space dipole-dipole interactions. For small molecules ( $MW < ca\ 2000$ ) the effect is usually positive and its magnitude is generally inversely proportional to the internuclear distance raised to the sixth power. Although conclusions based on internuclear distances obtained by quantitative analysis of NOE effects may be risky, the qualitative comparison of these effects within a molecule and between similar molecules (especially stereoisomers) can provide a solid basis for stereochemical assignment.<sup>181,182</sup>

In principle, a comparative study of the relative NOEs between the H2 and H3 proton resonances of the four diastereomers (**6.11**, **6.12**, **6.16a** and **6.16b**) could be used to determine their relative configuration. Unfortunately the very close proximity of these peaks in the spectra of three of the compounds precluded this type of experiment, since the required selective irradiation could not be achieved.

A NOESY (nuclear Overhauser effect spectroscopy)<sup>183</sup> experiment is a two dimensional method for simultaneously obtaining all interproton NOE interactions within a molecule.<sup>182,184</sup> The requirement for selective irradiation is avoided since all



resonances are simultaneously saturated with a non-selective  $90^\circ$  radio frequency pulse, making the method particularly useful for molecules with complex spectra.

NOESY spectra (500 MHz,  $\text{CDCl}_3$ ) were obtained for the four 6-hydroxybutanolide diastereomers (Figures 6.1-6.4) using a phase sensitive version of the experiment in which the cross-peaks due to NOE interactions appear with opposite phase (coloured red) to both the diagonal peaks and to spurious 'artefact' cross-peaks arising from the data processing (coloured blue/green). The same sample preparation techniques,<sup>181</sup> experimental parameters and data processing were used for all compounds, allowing valid comparisons to be drawn between the spectra.

Figure 6.1 NOESY NMR Spectrum (500 MHz,  $\text{CDCl}_3$ )  
of 6-Hydroxybutanolide 6.16a

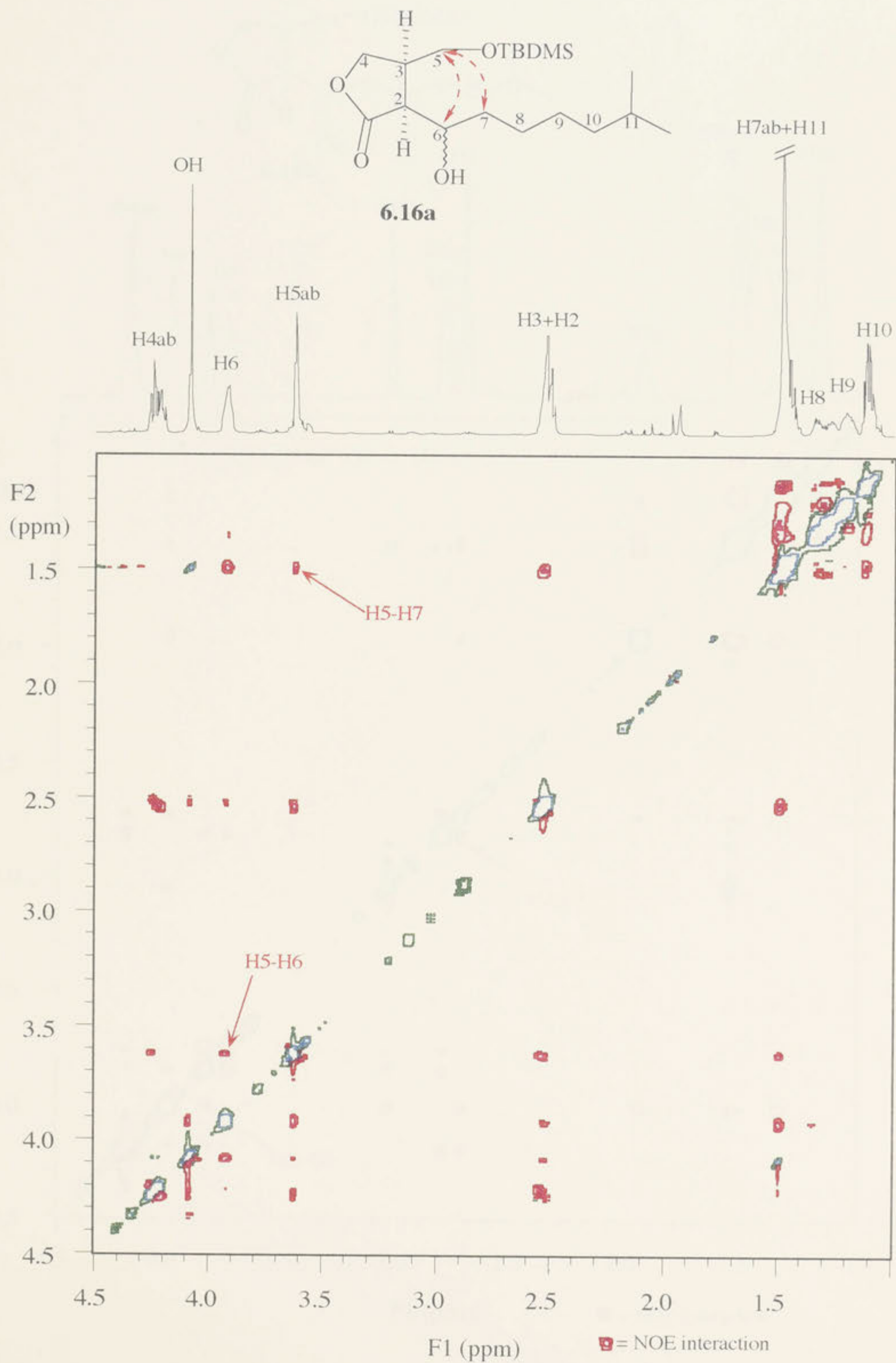




Figure 6.2 NOESY NMR Spectrum (500 MHz,  $\text{CDCl}_3$ )  
of 6-Hydroxybutanolide 6.16b

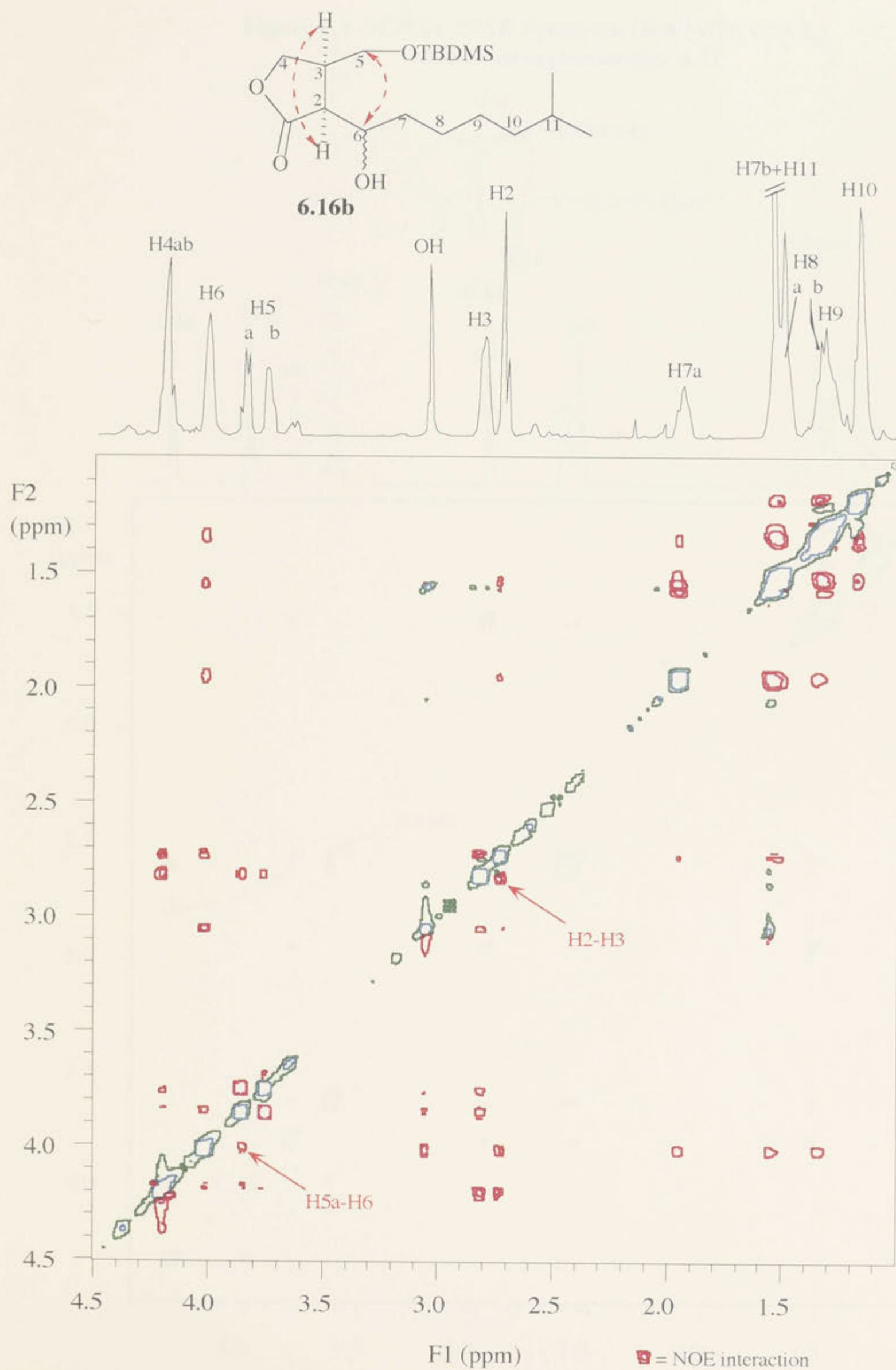


Figure 6.3 NOESY NMR Spectrum (500 MHz, CDCl<sub>3</sub>)  
of 6-Hydroxybutanolide 6.11

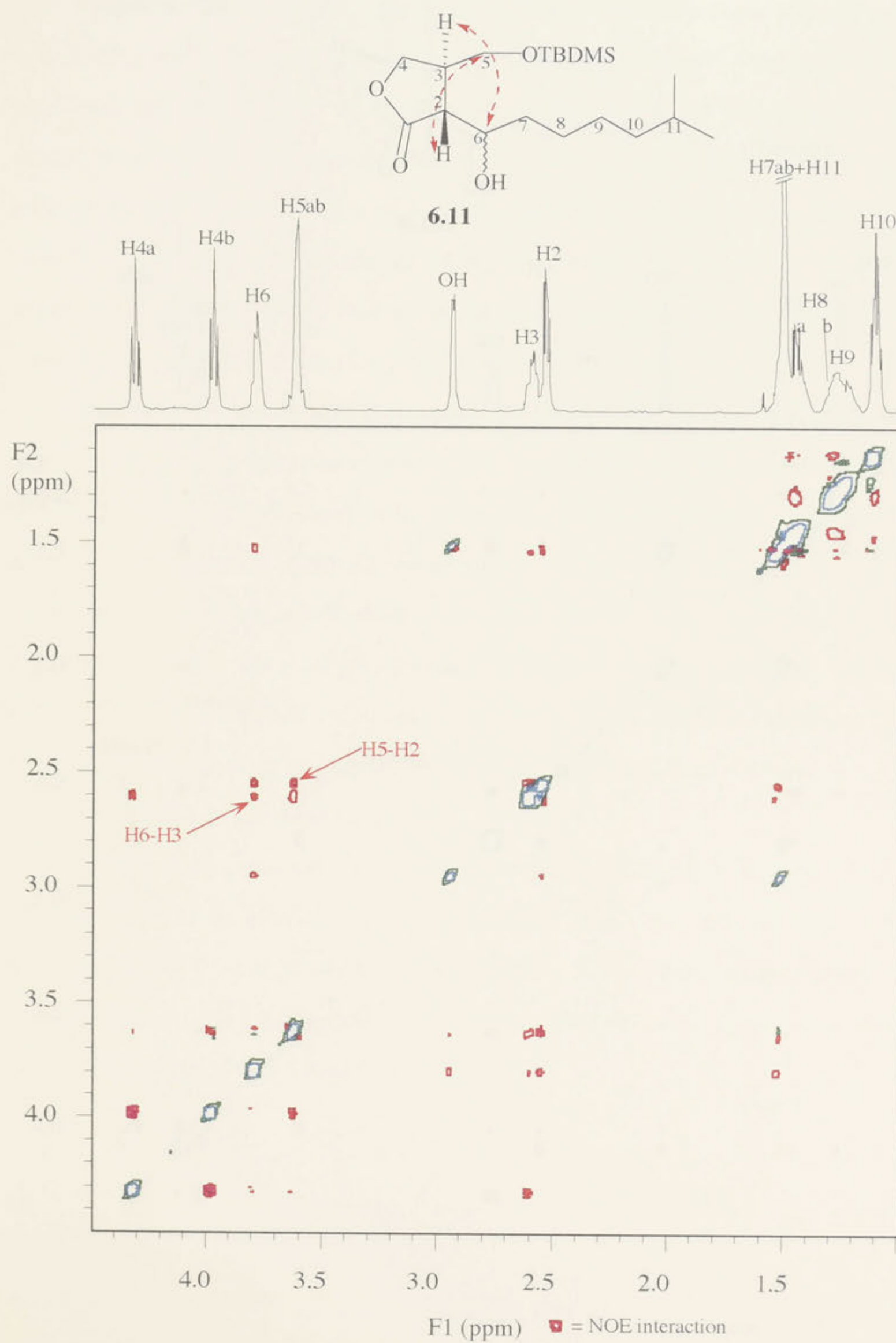
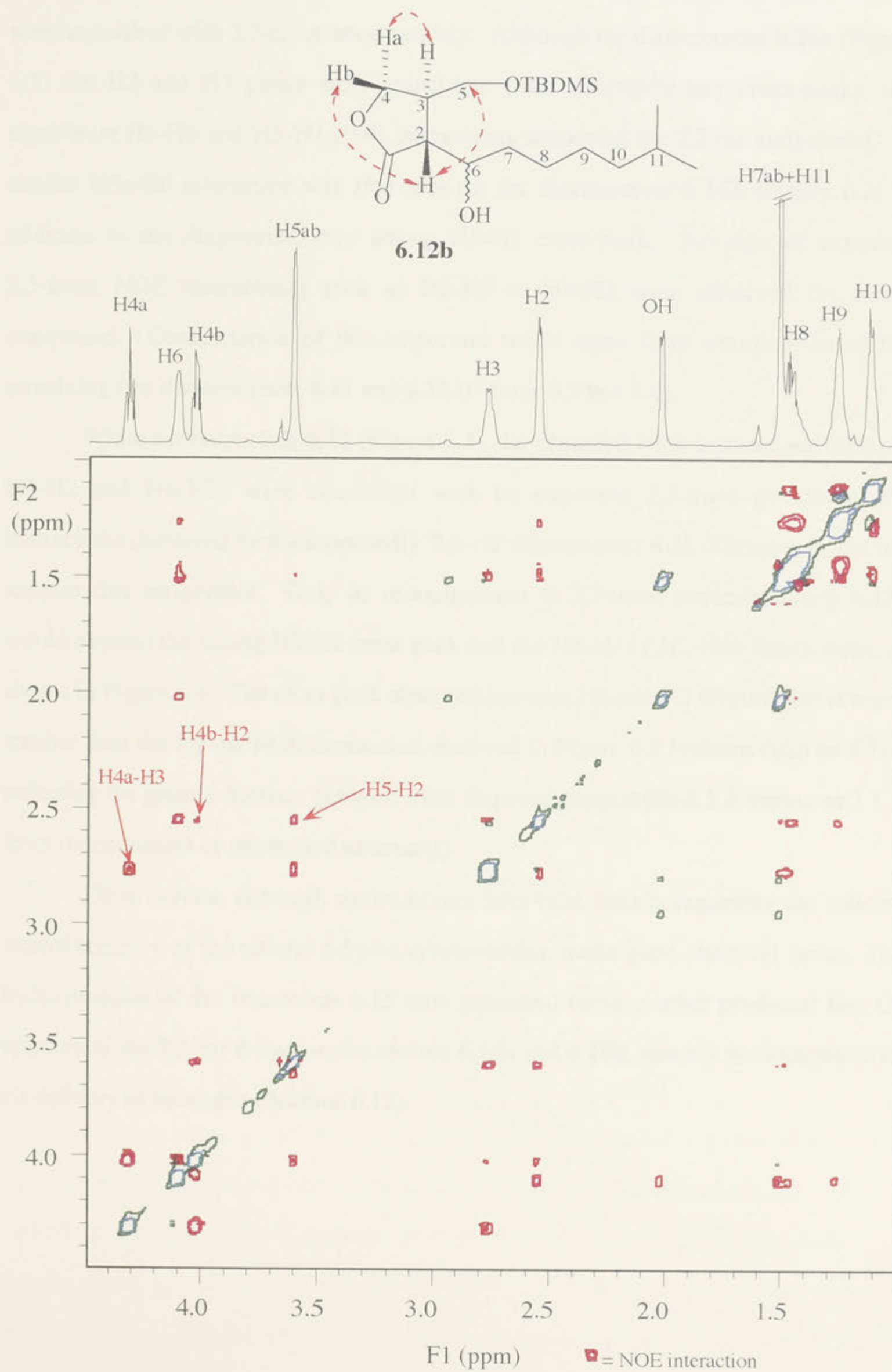




Figure 6.4 NOESY NMR Spectrum (500MHz, CDCl<sub>3</sub>)  
of 6-Hydroxybutanolide 6.12b

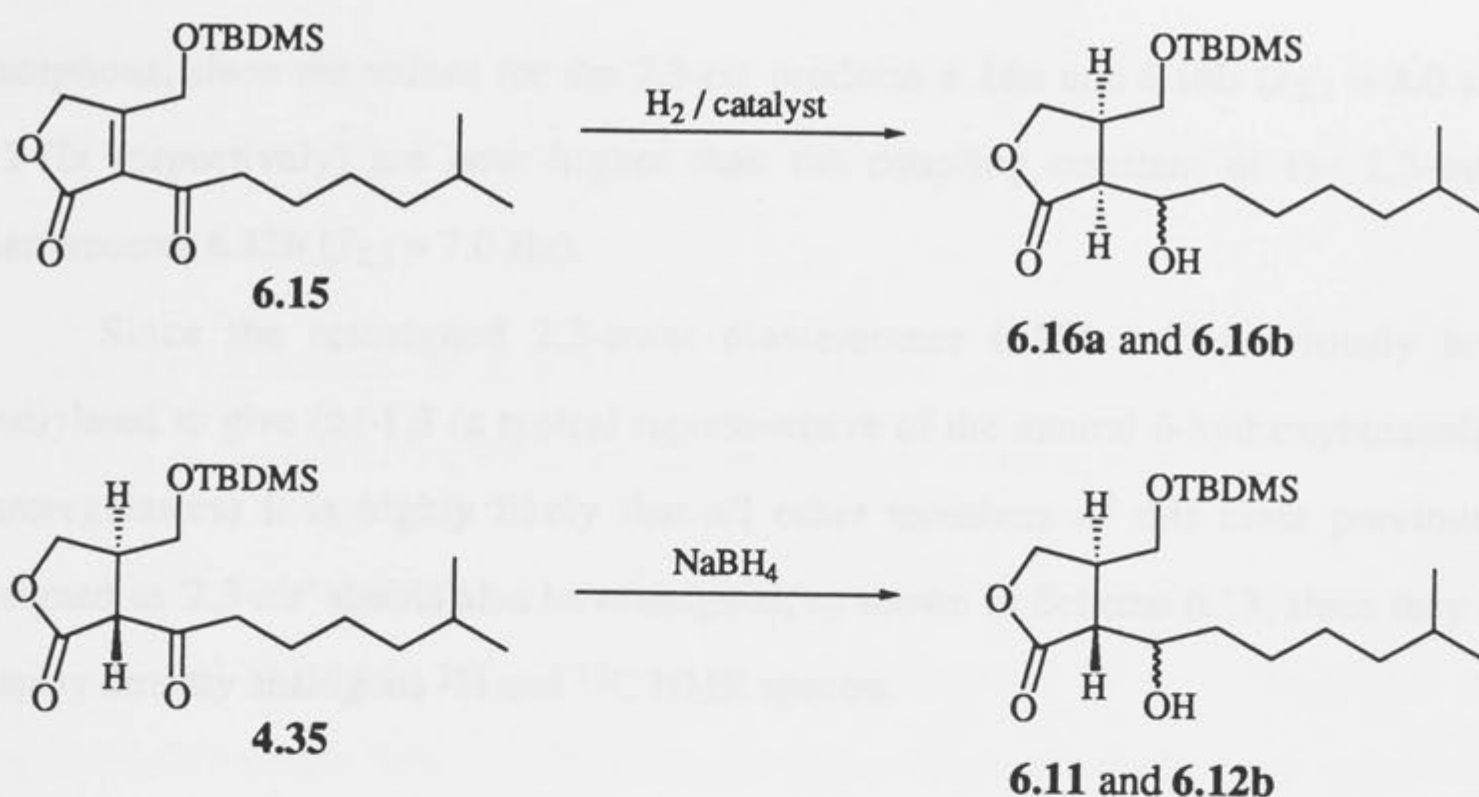


The NOESY spectra (Figures 6.1 and 6.2) of the two diastereomers **6.16a** and **6.16b**, prepared *via* catalytic hydrogenation, both displayed NOE interactions which were consistent with 2,3-*cis* stereochemistry. Although for diastereomer **6.16a** (Figure 6.1) the H2 and H3 peaks were coincident (thus obscuring any cross-peaks) the significant H5-H6 and H5-H7 NOE interactions supported the 2,3-*cis* assignment. A similar H5a-H6 interaction was also seen for the diastereomer **6.16b** (Figure 6.2) in addition to the diagnostic, very strong H2-H3 cross-peak. No sign of expected 2,3-*trans* NOE interactions such as H2-H5 or H6-H3 were observed for either compound. Confirmation of this important result came from examination of the remaining two diastereomers **6.11** and **6.12** (Figures 6.3 and 6.4).

While for butanolide **6.11** (Figure 6.3), the observed NOE interactions (notably H5-H2 and H6-H3) were consistent with its expected 2,3-*trans* geometry, the interactions displayed by the supposedly '2,3-*cis*' diastereomer **6.12** (Figure 6.4), did not support this assignment. Only its re-assignment to 2,3-*trans* stereochemistry **6.12b** would explain the strong H5-H2 cross peak and the H3-H4a / H2-H4b interactions, as shown in Figure 6.4. The cross peak observed between H2 and H3 (Figure 6.4) is much smaller than the 2,3-*cis* NOE interaction observed in Figure 6.2 (volume ratio *ca* 5.1:1) reflecting the greater distance between *trans* disposed protons (*ca* 2.3 Å versus *ca* 3.1 Å from measurement of minimised structures).

These results, although contradicting long-held beliefs regarding the relative stereochemistry of the natural 6-hydroxybutanolides, make good chemical sense. The hydrogenation of the butenolide **6.15** thus generated (among other products) two C6 epimers of the 2,3-*cis* 6-hydroxybutanolide **6.16a** and **6.16b**, exactly as expected from *cis* delivery of hydrogen (Scheme 6.12).





Scheme 6.12

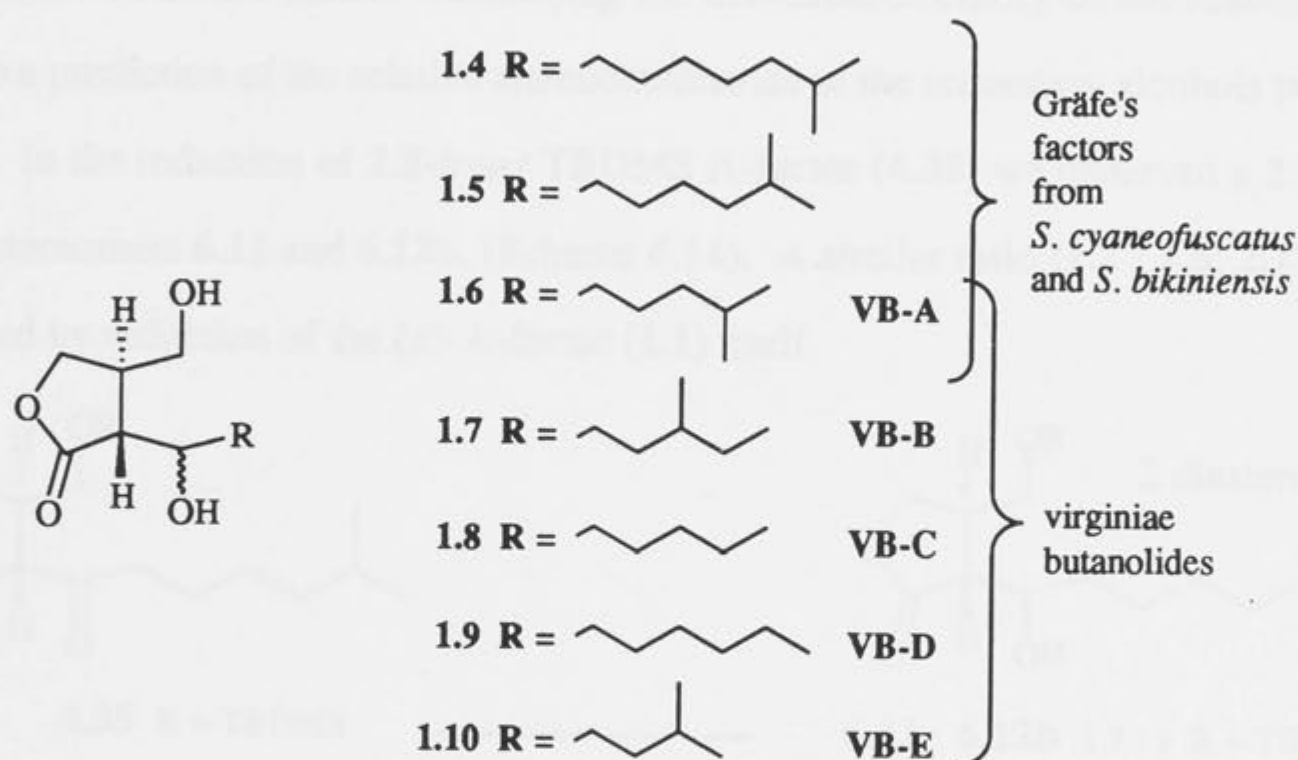
Similarly, the mystery surrounding the apparently unusual diastereoselectivity of the sodium borohydride reduction of TBDMS protected A-factor **4.35** evaporated when the 6-hydroxy products **6.11** and **6.12b** were identified simply as C6-hydroxy epimers which incorporate the 2,3-*trans* geometry of their ketone precursor **4.35**. However it was initially difficult to rationalise the reassignment of 2,3-*trans* geometry to both borohydride reduction products with the reported conversion of one of them (allegedly '2,3-*cis*' **6.5**) into a *cis* fused bicyclic compound **6.6** (or its C6 epimer), by Mori and Chiba (see Scheme 6.2).<sup>30</sup> It now appears likely that during the prolonged treatment with hot pyridine (100°, 5 h) some C2 epimerisation of the (presumably 2,3-*trans*) butanolide occurred, allowing cyclisation to occur, to form the observed moderate yield (45%) of 2,3-*cis* bicyclic product **6.6**. Alternatively, preliminary translaconisation of the 2,3-*trans* **6.5** (acid or base catalysed in *p*-TsCl / pyridine) would also produce a 2,3-*cis* butanolide capable of forming some of a *cis* bicyclic product.

With this 2,3-stereochemical reassignment the supposed empirical relationship between the  $J_{2,3}$  coupling constant and C2, C3 relative stereochemistry in  $\gamma$ -lactones (which previously had been universally applied to the stereochemical assignment for 6-hydroxybutanolide autoregulators)<sup>24-26,28,30,35</sup> was exposed as being dangerously unreliable. Even the simple 'rule' that 2,3-*trans* diastereomers have higher 2,3-vicinal coupling values than the equivalent 2,3-*cis* compounds<sup>157</sup> was shown to have



exceptions, since the values for the 2,3-*cis* products **6.16a** and **6.16b** ( $J_{2,3} = 8.0$  and 7.2 Hz respectively) are both higher than the coupling constant of the 2,3-*trans* diastereomer **6.12b** ( $J_{2,3} = 7.0$  Hz).

Since the reassigned 2,3-*trans* diastereomer **6.12b** had previously been desilylated to give ( $\pm$ )-**1.5** (a typical representative of the natural 6-hydroxybutanolide autoregulators) it is highly likely that all other members of this class previously assigned as '2,3-*cis*' should also be reassigned, as shown in Scheme 6.13, since they all display directly analogous  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra.



**Scheme 6.13 Reassigned 2,3-Stereochemistry of the Former '2,3-*cis*' 6-Hydroxybutanolides**

The increased activity exhibited by this class of compounds which was previously attributed to their 2,3-*cis* geometry<sup>35</sup> is now shown to result from their C6 hydroxyl stereochemistry. Since the two methods used by Mori and Chiba<sup>30</sup> to attempt to define this configuration are likely to be erroneous (see Section 6.2.3) we have used an alternative approach to this problem (Section 6.4.2).

It was ironic that although we had successfully achieved our goal of developing a diastereospecific synthesis of the 2,3-*cis* 6-hydroxybutanolides, our work exposed errors in the previous stereochemical assignment of the target class of autoregulators,

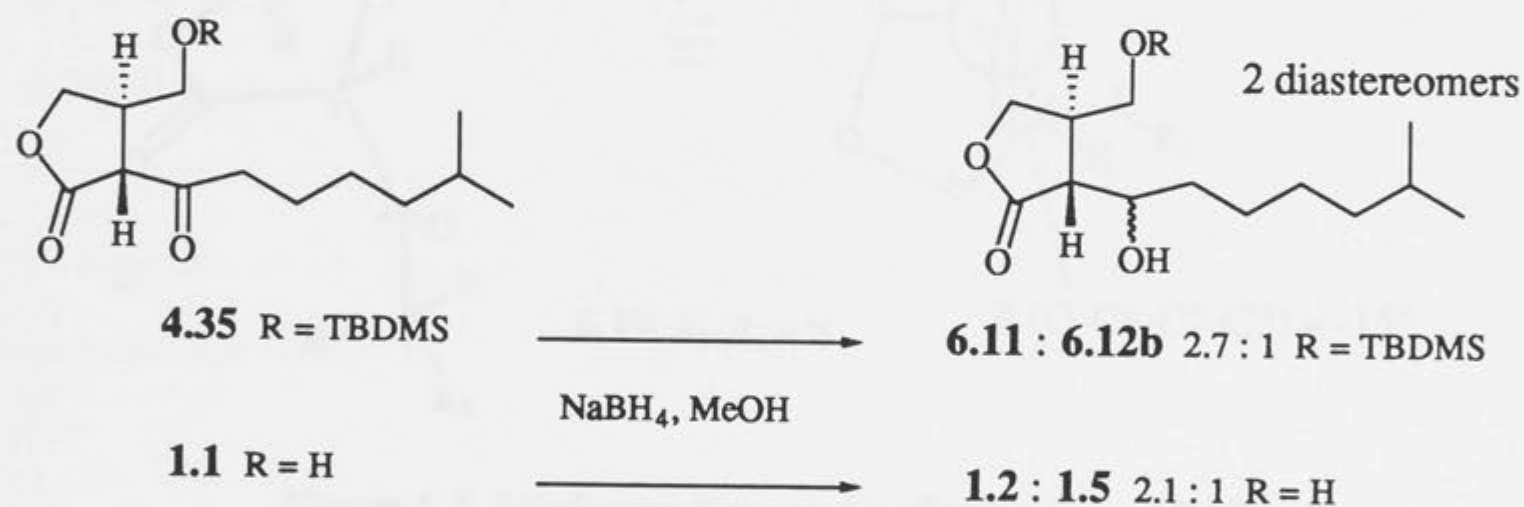


which removed the original need for the synthesis. However, our synthesis does provide access to a novel class of butanolides with as yet unknown biological activity.

#### 6.4.2 Prediction of C6 Configuration of the 6-Hydroxybutanolide Autoregulators

With our clarification of the 2,3-stereochemical outcome of the sodium borohydride reduction of 2,3-*trans*-6-ketobutanolides, it was proposed that rationalisation of the factors influencing the diastereoselectivity of the reaction could lead to a prediction of the relative stereochemistries of the secondary alcohols produced.

In the reduction of 2,3-*trans* TBDMS A-factor (4.35) we observed a 2.7:1 ratio of diastereomers 6.11 and 6.12b, (Scheme 6.14). A similar ratio (1.2 : 1.5, 2.1 : 1) was obtained by reduction of the ( $\pm$ )-A-factor (1.1) itself.



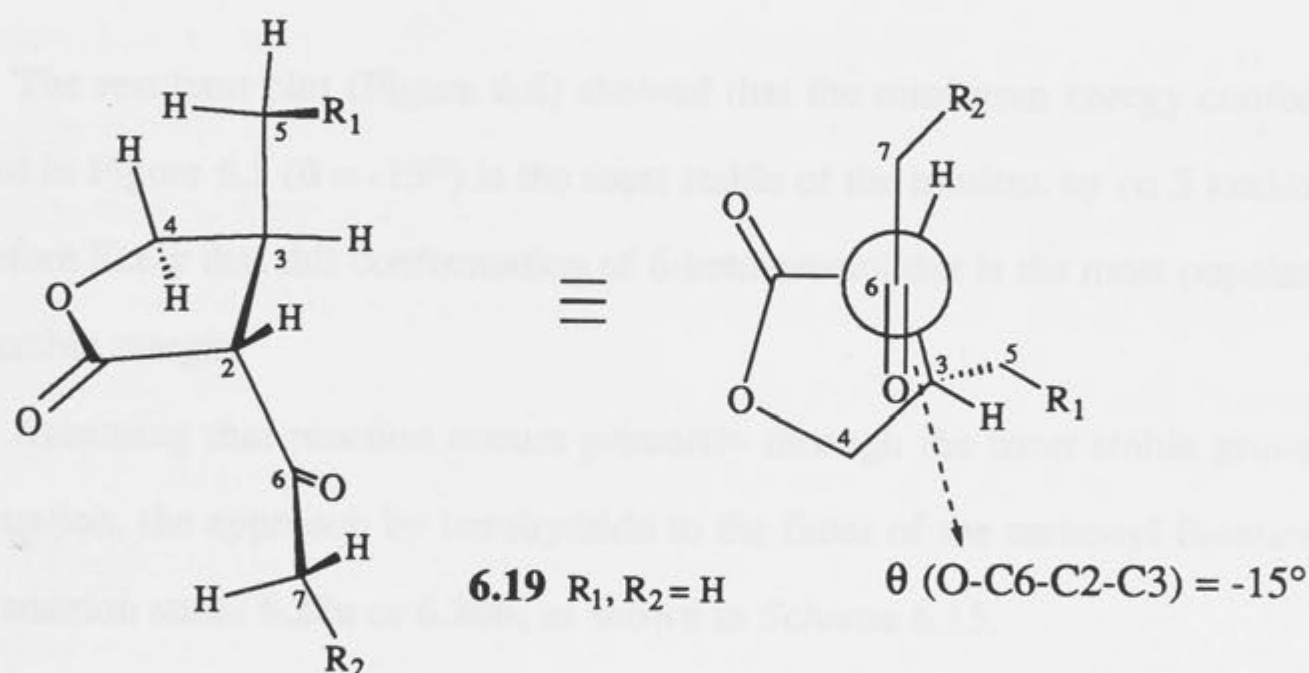
Scheme 6.14

The unequal formation of the C6 epimers during reduction clearly reflects a preference for attack by borohydride on one face of the ketone moiety due to the asymmetry of the rest of the molecule.

This type of 1,2-asymmetric induction involving the attack of nucleophilic reagents on chiral aldehydes or ketones has been well studied<sup>185-187</sup> and the stereochemical outcome has been found to be strongly influenced by steric effects on the attacking nucleophile. These reactions are generally accepted as being under 'steric approach control' which implies an early 'reactant-like' transition state.<sup>186,187</sup> It was

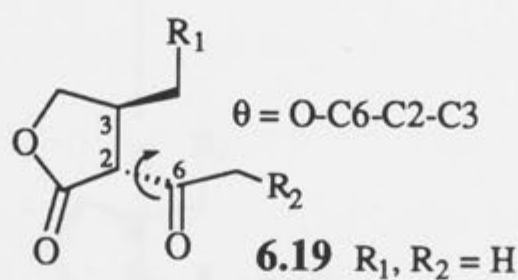
thus important to our consideration of competing transition states to have information regarding the preferred conformation of the 6-keto group of the substrate.

We performed MM2 energy minimisations (PC Model, version 88.0) using the simplified model structure **6.19** to represent the general class of 2,3-*trans* disubstituted-2-acyl butanolides. The minimum energy conformation was found to have the ketone C=O bond almost *syn*-periplanar to the C2-C3 bond (torsion angle  $\theta = \text{O}-\text{C6}-\text{C2}-\text{C3} = -15^\circ$ ), as depicted in Fig 6.5. In this conformation the steric interaction of C7 is minimised, a factor which is of major importance in the highly regarded<sup>188</sup> Felkin model of the required transition state geometry for nucleophilic attack on chiral ketones ( see Scheme 6.15).<sup>186</sup>

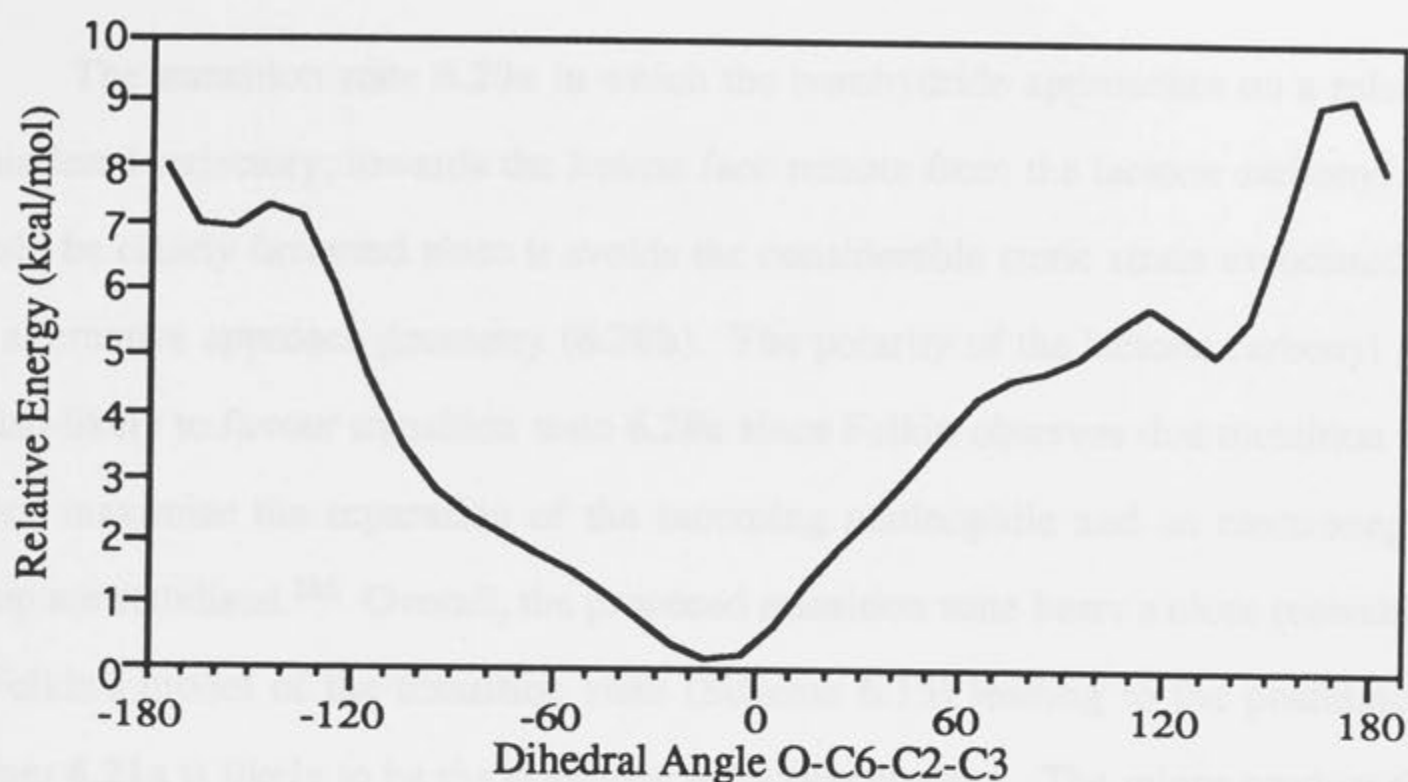


**Figure 6.5** Minimum Energy Conformation of **6.19**

The relative conformational energies of other orientations of the 6-keto group were determined by repeatedly calculating the energy of the structure **6.19** as the C2-C6 bond was rotated stepwise.



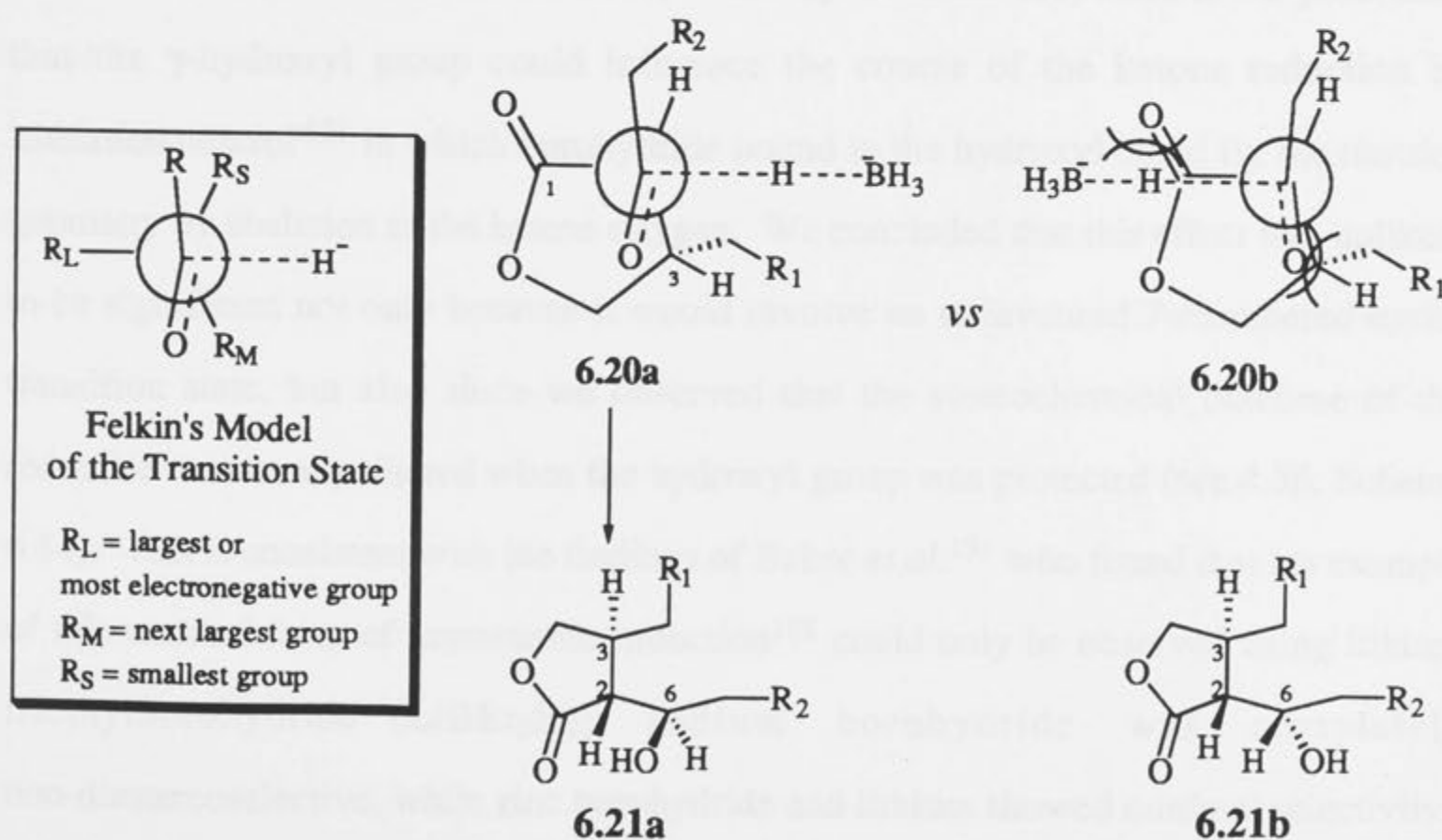




**Figure 6.6** Energy Profile of 6.19 for Rotation about the C2-C6 Bond

The resultant plot (Figure 6.6) showed that the minimum energy conformation depicted in Figure 6.5 ( $\theta = -15^\circ$ ) is the most stable of the minima by *ca* 5 kcal/mole. It is therefore likely that this conformation of 6-ketobutanolides is the most populated by a considerable margin.

Assuming that reaction occurs primarily through the most stable ground-state conformation, the approach by borohydride to the faces of the carbonyl function could form transition states **6.20a** or **6.20b**, as shown in Scheme 6.15.



**Scheme 6.15**



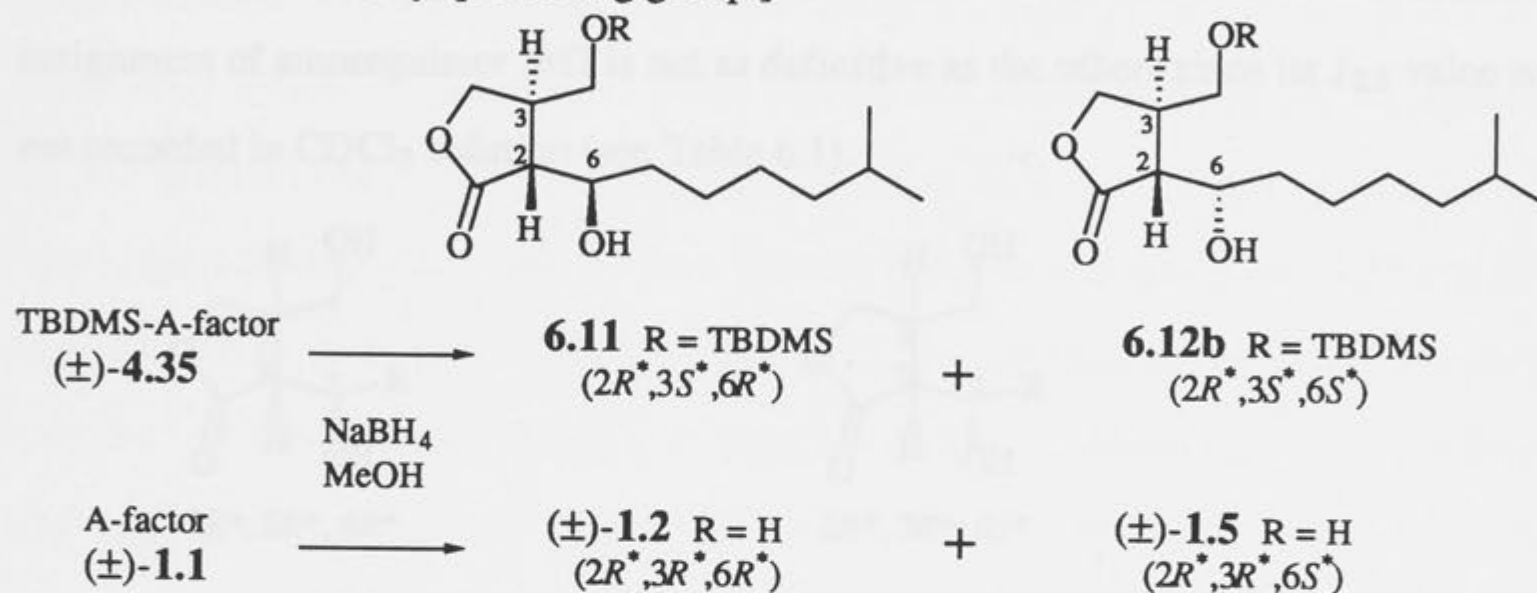
The transition state **6.20a** in which the borohydride approaches on a relatively unhindered trajectory, towards the ketone face remote from the lactone carbonyl (C1), would be clearly favoured since it avoids the considerable steric strain associated with the alternative approach geometry (**6.20b**). The polarity of the lactone carbonyl group is also likely to favour transition state **6.20a** since Felkin observes that transition states which maximise the separation of the incoming nucleophile and an electronegative group are stabilised.<sup>186</sup> Overall, the proposed transition state bears a close resemblance to Felkin's model of the transition state (Scheme 6.15) leading to the postulate that epimer **6.21a** is likely to be the dominant reduction product. The minor product **6.21b** could result either from the disfavoured transition state **6.20b** or *via* reaction through an alternative, higher energy conformation of the substrate **6.19**.

The sodium counter ion has not been mentioned in the above discussion since it is not expected to have a significant influence on the transition state. Wigfield and Gowland<sup>189</sup> observe that "*Although in some related reductions the cation does play an important role, there is no evidence for a role for Na<sup>+</sup> in NaBH<sub>4</sub> reductions in alcohol*". For example, the addition of crown ethers to NaBH<sub>4</sub> reductions in methanol has been found to have no effect on the reactions.<sup>190</sup>

For substrates **6.19** in which R<sub>1</sub> = OH (*cf.* A-factor **1.1**) there is the possibility that the  $\gamma$ -hydroxyl group could influence the course of the ketone reduction by 'chelation control'<sup>191</sup> in which borohydride bound to the hydroxyl could fix the reaction geometry by chelation to the ketone oxygen. We concluded that this effect was unlikely to be significant not only because it would involve an unfavoured 7-membered cyclic transition state, but also since we observed that the stereochemical outcome of the reduction was barely altered when the hydroxyl group was protected (see **4.35**, Scheme 6.14). This is consistent with the findings of Baker *et al.*<sup>191</sup> who found that his example of this unusual form of asymmetric induction<sup>188</sup> could only be observed using lithium triethylborohydride (LiBEt<sub>3</sub>H); sodium borohydride was completely non-diastereoselective, while zinc borohydride and lithium showed minimal selectivity.



Therefore, with the assumption that the 6-ketobutanolide **4.35** reacts primarily *via* its most stable C2-C6 conformation (*cf.* **6.19**, Figure 6.5), we propose that the major product **6.11** from its reduction with sodium borohydride is likely to have  $2R^*$ ,  $3S^*$ ,  $6R^*$  configuration (*cf.* **6.21a**) while for the minor diastereomer **6.12b**  $2R^*$ ,  $3S^*$ ,  $6S^*$  geometry is predicted (*cf.* **6.21b**), as shown in Scheme 6.16. Analogous assignments can also be made for the ( $\pm$ )-A-factor reduction products ( $\pm$ )-**1.2** and ( $\pm$ )-**1.5** (Scheme 6.16). [Note again that the stereochemical designation for C3 reverses with removal of the silyl protecting group.]



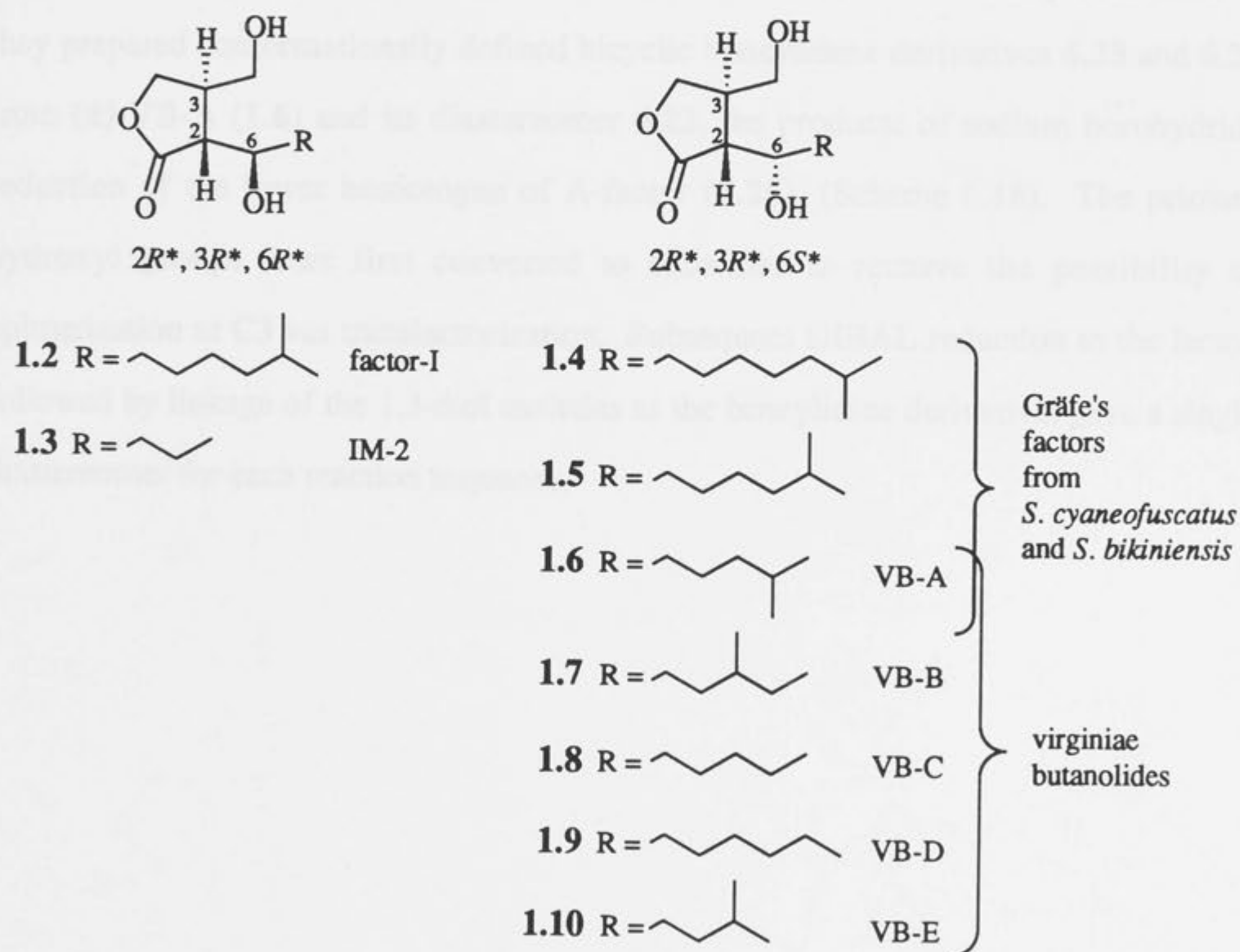
**Scheme 6.16 Fully Reassigned Stereochemistry of the  $\text{NaBH}_4$  Reduction Products**

These predictions of C6 configurations conflict with the recent assignment by Mori and Chiba<sup>30</sup> but are consistent with our previously discussed reassessment of his evidence (Section 6.2.3).

We were not able to use the NOESY spectra of **6.11** and **6.12b** (Figures 6.3 and 6.4) to provide confirmation of our predicted C6 configurations due to uncertainties regarding their precise molecular geometry in solution. Similarly, we could not assign the C6 geometry of the 2,3-cis 6-hydroxybutanolides **6.16a** and **6.16b** on the basis of their NOESY spectra (Figures 6.1 and 6.2). In addition, since no diastereoselectivity was observed in their formation by catalytic hydrogenation, arguments based on asymmetric induction could not be employed.

### 6.4.3 Full Stereochemical Reassignment of the 6-Hydroxybutanolide Autoregulators

Since the compounds **1.2** and **1.5** are representative of the two diastereomeric classes of naturally occurring 2,3-*trans* 6-hydroxybutanolide autoregulators, it is possible for us to predict the complete relative stereochemistry of all of the natural metabolites (Scheme 6.17), based upon the close similarity of their crucial  $^1\text{H}$  NMR resonances to those of either **1.2** or **1.5** (see Table 6.1). Note that the indicated assignment of autoregulator IM2 is not as definitive as the others since its  $J_{2,3}$  value was not recorded in  $\text{CDCl}_3$  solution (see Table 6.1).



**Scheme 6.17 Predicted Relative Configuration for All 6-Hydroxybutanolide Autoregulators**

From biosynthetic considerations it is thought likely that all of these closely related autoregulators share the  $3R$  configuration eventually established by Mori for A-factor (**1.1**)<sup>18</sup> and for three of the 6-hydroxybutanolides (**1.6**, **1.7** and **1.8**).<sup>30</sup> Thus

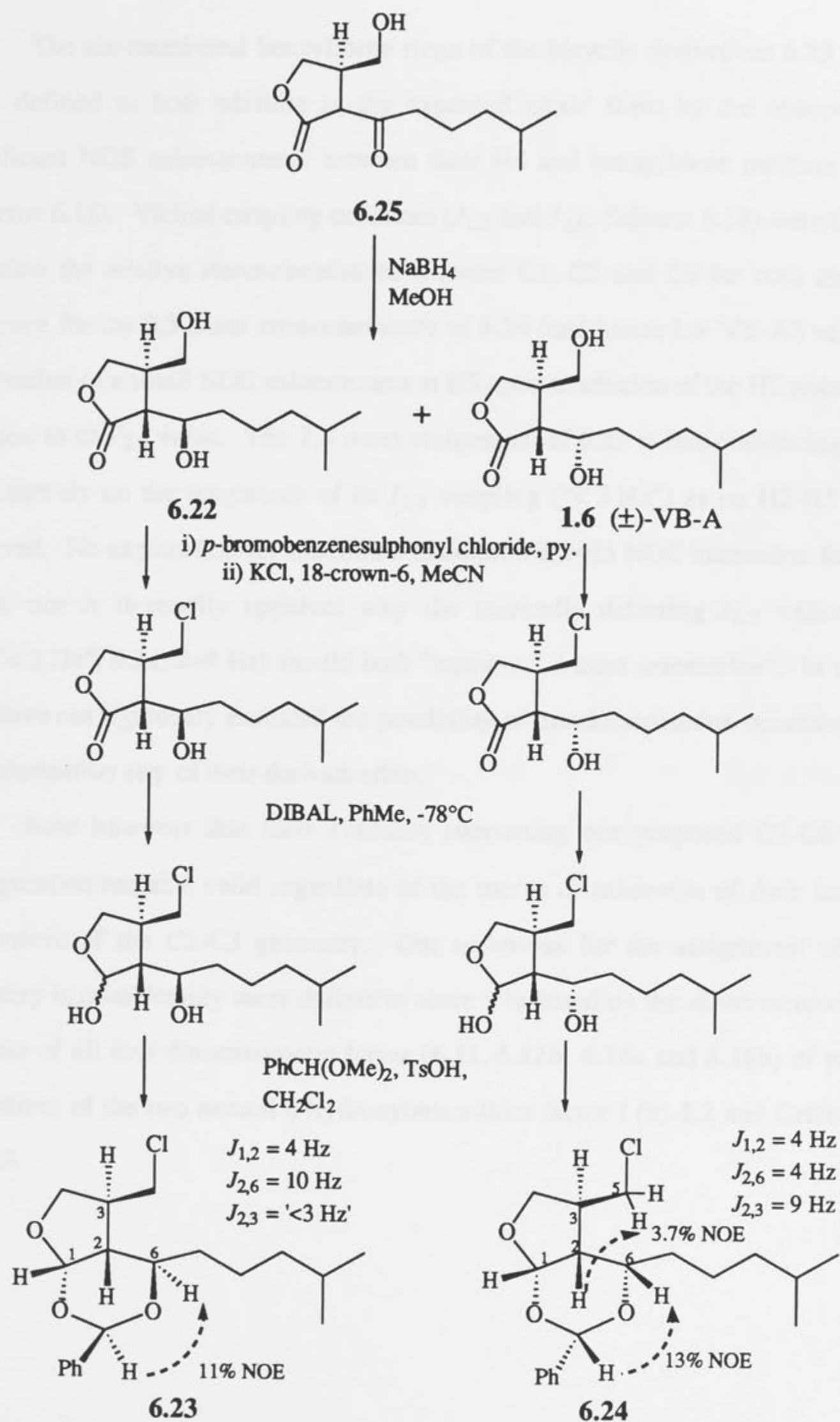


the predicted structures as drawn in Scheme 6.17 are likely also to represent the *absolute* configurations of the natural butanolide autoregulators.

#### CONFIRMATION OF THE STEREOCHEMICAL REASSIGNMENTS

Shortly after these predictions were made, our efforts towards their confirmation were arrested by the appearance of a report by Sakuda and Yamada in which identical stereochemical conclusions were drawn using an entirely different approach.<sup>27</sup> Apparently alerted by anomalous NOE results that their previous assignment<sup>25</sup> of '2,3-*cis*' geometry to the virginiae butanolides (VB-A to E, 1.6-1.10) may be in error<sup>24</sup>, they prepared conformationally defined bicyclic benzylidene derivatives 6.23 and 6.24 from (±)-VB-A (1.6) and its diastereomer 6.22, the products of sodium borohydride reduction of the lower homologue of A-factor (6.25), (Scheme 6.18). The primary hydroxyl groups were first converted to chlorides to remove the possibility of epimerisation at C3 *via* translactonisation. Subsequent DIBAL reduction to the lactol, followed by linkage of the 1,3-diol moieties as the benzylidene derivative, gave a single diastereomer for each reaction sequence.

SCHEME 6.18 Synthesis of Bicyclic Benzylidene Derivatives by Sakuda and Yamada



SCHEME 6.18 Synthesis of Benzylidene Derivatives by Sakuda and Yamada



The six-membered benzyldiene rings of the bicyclic derivatives **6.23** and **6.24** were defined as both existing in the expected 'chair' form by the observation of significant NOE enhancements between their H6 and benzyldiene methine protons, (Scheme 6.18). Vicinal coupling constants ( $J_{1,2}$  and  $J_{2,6}$ , Scheme 6.18) were then used to define the relative stereochemistries between C1, C2 and C6 for both molecules. Evidence for the 2,3-*trans* stereochemistry of **6.24** (and hence **1.6** 'VB-A') came from observation of a small NOE enhancement at H5 upon irradiation of the H2 resonance, in addition to its  $J_{2,3}$  value. The 2,3-*trans* assignment of **6.23** is less convincing since it rests entirely on the magnitude of its  $J_{2,3}$  coupling (" $< 3$  Hz") as no H2-H5 NOE is observed. No explanation for the failure to detect a H2-H5 NOE interaction for **6.23** is given, nor is it readily apparent why the markedly differing  $J_{2,3}$  values (**6.23**,  $J_{2,3}$  = " $< 3$  Hz"; **6.24**,  $J$  = 9 Hz) should both "*support 2,3-trans orientation*". In addition, they have not rigorously excluded the possibility of translactonisation occurring during the chlorination step of their derivatisation.

Note however that their evidence supporting our proposed C2-C6 relative configuration remains valid regardless of the merits or otherwise of their method of assignment of the C2-C3 geometry. Our technique for the assignment of C2-C3 geometry is considerably more definitive since it is based on the *direct* stereochemical analysis of all four diastereomeric forms (**6.11**, **6.12b**, **6.16a** and **6.16b**) of protected derivatives of the two natural 6-hydroxybutanolides factor-I ( $\pm$ )-**1.2** and Gräfe's factor ( $\pm$ )-**1.5**.

## 6.5 CONCLUDING COMMENTS

Although starting in this area with intentions to improve the synthesis of the supposed '2,3-*cis*' 6-hydroxybutanolide autoregulators, our work in this area concludes with the reassignment of two of the three chiral centres of the target class of compounds, which are the most biologically active and numerous group of these autoregulators (1.4-1.10). Our work has redefined the synthetic task as the formation of the required C6 epimer of a 2,3-*trans* 6-hydroxybutanolide.

Yamada's group has recently had some success in this pursuit. Sakuda and Yamada<sup>27</sup> have shown that derivatives of VB-A(1.6) are available from the less active but more readily available C6 epimer (6.22, Scheme 6.18) *via* epimerisation using the Mitsunobu reaction.<sup>192</sup> The enzymatic reduction of 6-ketobutanolide (6.25) to VB-A (1.6) has also apparently been very recently achieved,<sup>160</sup> using cell-free extracts of *S. antibioticus* in the presence of NADPH, but no further details of the efficiency or specificity of this process are yet available.

Rather than entering this area of synthesis we instead turned our attention to a more challenging target with which to test the scope and limitations of our biomimetic route to 2,3-disubstituted butanolides.



## CHAPTER SEVEN

### A MODEL BIOMIMETIC SYNTHESIS OF BUTALACTIN:

### A BUTANOLIDE ANTIBIOTIC

*Round about the cauldron go;  
In the poison'd entrails throw.*

William Shakespeare (1564-1616)

Macbeth, Act IV, Scene 1

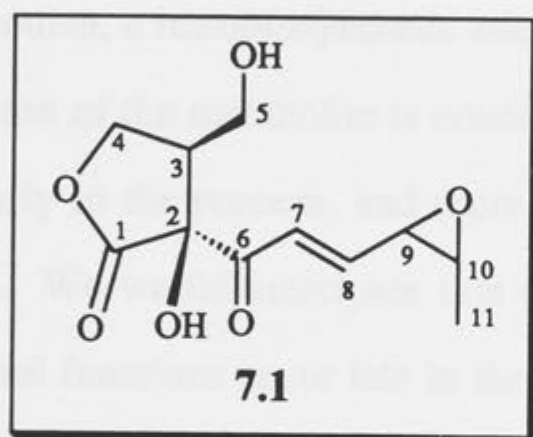
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## 7.1 INTRODUCTION

In 1991 Franco and coworkers reported the isolation of a novel  $\gamma$ -lactone metabolite named 'butalactin' (**7.1**) from *Streptomyces* sp. Y-86,36923.<sup>193</sup> Its structure<sup>194</sup> was found to have much in common with A-factor (**1.1**), such as the *trans* disposed 3-hydroxymethyl and 2-acyl substituents, but incorporated several novel features: the 2-hydroxyl group, the conjugated *trans* olefin and the 9-*cis*-epoxide. However, despite the structural similarities with A-factor, their occurrence and biological activities differ markedly.



### butalactin

- from *Streptomyces* sp. Y-86,36923
- antibiotic activity against Gram positive bacteria
- no known inducing properties
- weak *inhibitor* of glycopeptide antibiotic production

The metabolite is present in the culture broth at a level of *ca* 40 mg/L which is 3-4 orders of magnitude higher than levels of A-factor (**1.1**) and related autoregulators in their cultures. Unlike A-factor butalactin does not induce antibiotic production or morphological changes in *S. griseus*, even at 100 times the usual concentration<sup>194</sup> (though given the species specificity of the autoregulators this is not entirely surprising). It has no effect on the production of antibiotics of a wide range of producing strains, except for a weak inhibitory effect on the production of glycopeptide antibiotics of the vancomycin class. Remarkably, it also exhibits its own moderate antibiotic activity against Gram positive bacteria and has been patented for use as such.<sup>21</sup>

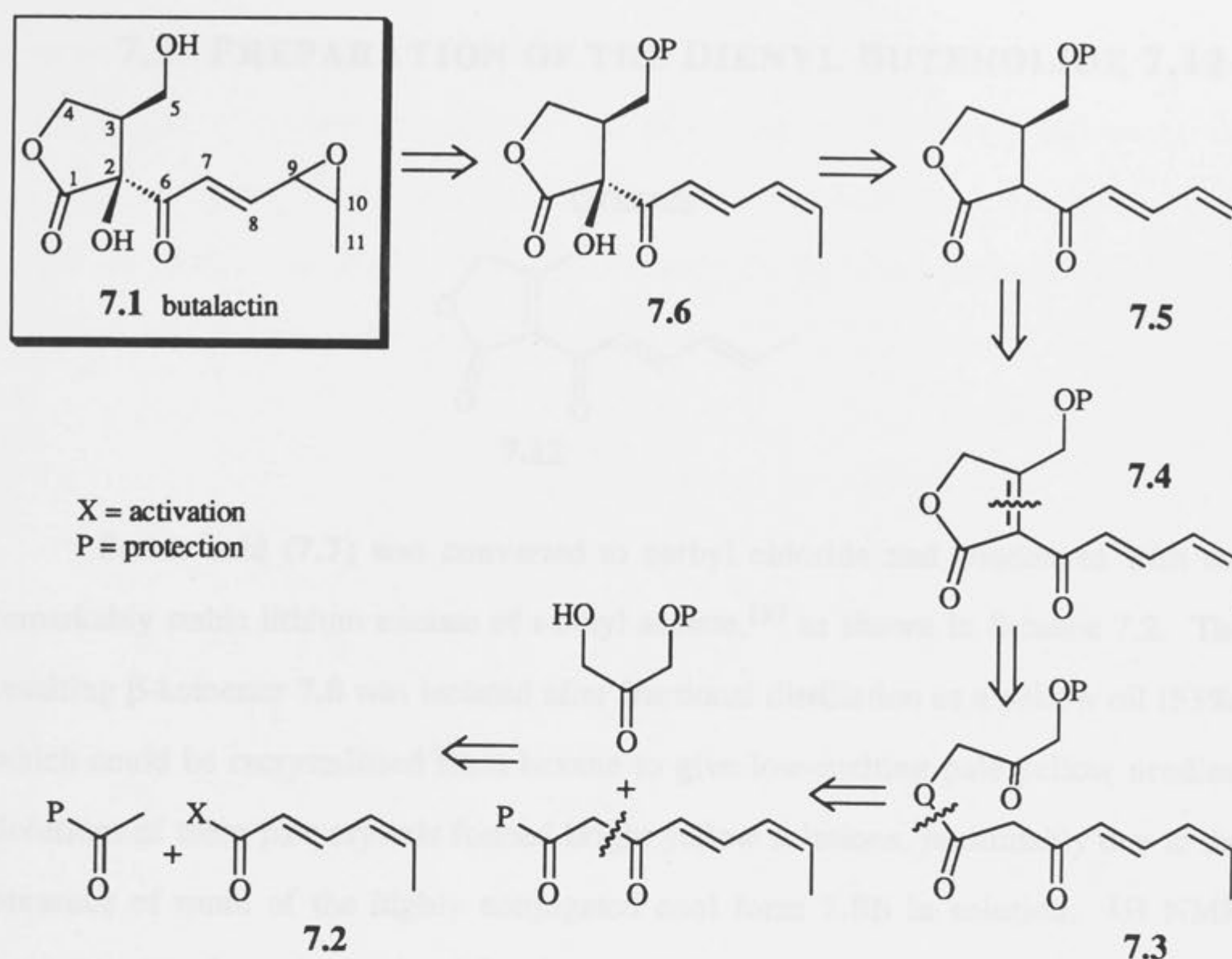
To date this interesting metabolite has not been synthesised and no information exists regarding its absolute configuration, its biosynthesis or the stereochemistry of the *cis*-epoxide moiety relative to that of the lactone substituents.

## 7.2 SYNTHETIC PLAN

As a synthetic target the molecule presents several additional challenges to those encountered during the synthesis of A-factor due to its increased functionality, and therefore represents a good test of the versatility of our biomimetic route to 2,3-substituted butyrolactones. We thus embarked on a brief investigation into the applicability of our biomimetic route to the synthesis of butalactin (7.1).

The retrosynthetic plan depicted in Scheme 7.1 is based on our synthetic route to A-factor and highlights the chemical transformations required in addition to the pivotal Knoevenagel cyclisation of the dienoic  $\beta$ -ketoester 7.3 to dienoic butenolide 7.4. It also reflects, of course, a *retrobiosynthetic* analysis of butalactin (7.1), at least as far as the carbon skeleton of the metabolite is concerned. It is likely that the diene functionality originates early in the process, and represents incomplete reduction during fatty acid biosynthesis. We would anticipate that the oxidation steps introducing epoxide and tertiary alcohol functions occur late in the process, but their relative timing is arbitrary and maybe the reverse of that depicted.





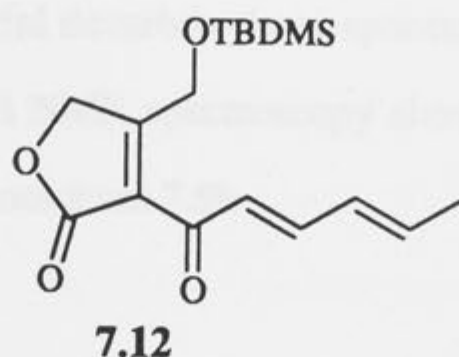
Scheme 7.1 Retrosynthesis of Butalactin

If the butenolide **7.4** could be prepared using chemistry analogous to that used for the synthesis of A-factor, we then would require:-

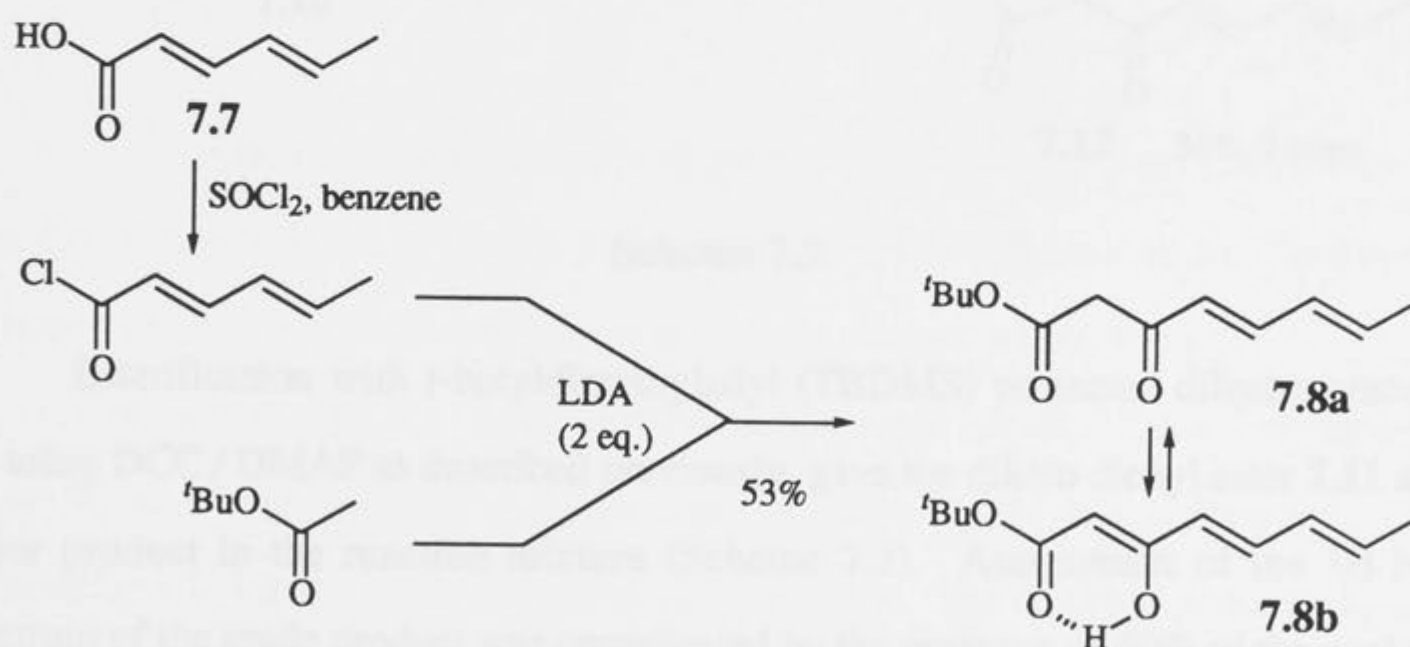
- Regiospecific reduction of the 2,3-bond in butenolide **7.4**, in the presence of a diene moiety.
- Diastereoselective C2-hydroxylation of the  $\beta$ -ketolactone **7.5**.
- Regiospecific epoxidation of the 9-*cis*-olefin **7.6**.

However, despite advances in synthesis of 2*E*,4*Z*-dienoic esters<sup>195,196</sup> they remain far less accessible than their *E,E*-analogues. In exploring the feasibility of the preceding synthetic plan it was thus prudent to use the inexpensive and readily available *E,E*-hexa-2,4-dienoic acid (**7.7**, sorbic acid) as the starting material (*cf.* **7.2**) for a model synthesis. The terminal *E*-olefinic bond could in principle be subsequently converted to a *trans*- or a *cis*-epoxide by direct epoxidation or by a two-step epoxidation involving inversion, respectively. Thus the synthesis of the *E,E*-analogue (**7.12**) of the *E,Z*-diene **7.4** was our initial synthetic objective.

## 7.3 PREPARATION OF THE DIENYL BUTENOLIDE 7.12



Sorbic acid (**7.7**) was converted to sorbyl chloride and condensed with the remarkably stable lithium enolate of *t*-butyl acetate,<sup>197</sup> as shown in Scheme 7.2. The resulting  $\beta$ -ketoester **7.8** was isolated after fractional distillation as a yellow oil (53%) which could be recrystallised from hexane to give low-melting pale yellow needles. Solutions of these pale crystals formed bright yellow solutions, presumably due to the presence of more of the highly conjugated enol form **7.8b** in solution.  $^1\text{H}$  NMR spectroscopy showed that in chloroform solution *ca.* 23% of the compound was enolised compared with 6% enol for *t*-butyl 3-oxononanoate (**3.33d**), thus demonstrating that the presence of the conjugated diene moiety gave extra stability to the enol form.

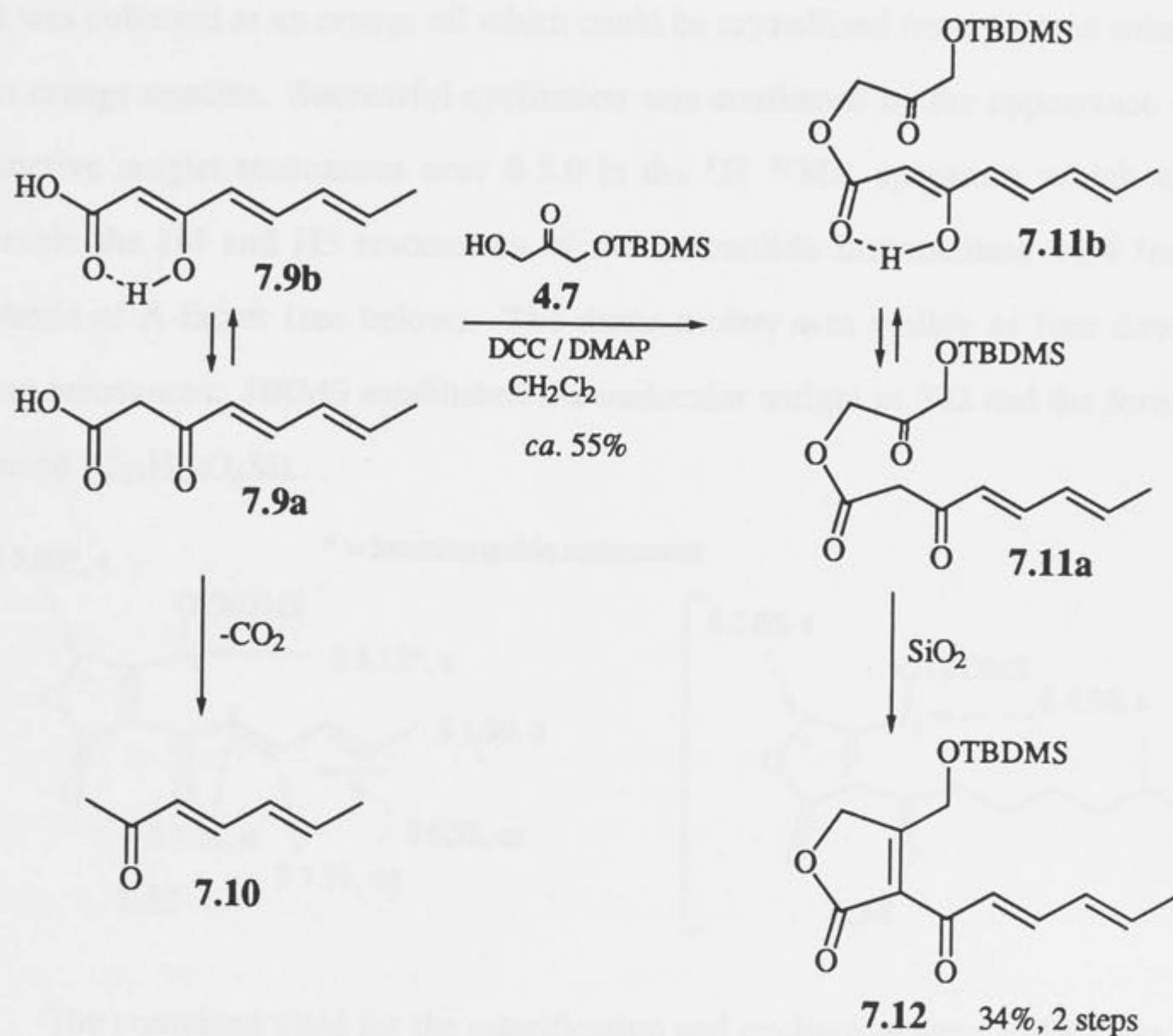


Scheme 7.2

The *t*-butyl  $\beta$ -ketoester **7.8** was converted to the  $\beta$ -ketoacid **7.9** using trifluoroacetic acid as described for the synthesis of A-factor, to give a 50% yield of pale yellow needles. Additional product was detected in the mother liquors but it



resisted crystallisation due to the presence of impurities, most notably the methyl ketone **7.10** formed by decarboxylation. As we had observed for other  $\beta$ -ketoesters, the impure, non-crystalline material decarboxylates spontaneously on standing for several hours. In  $\text{CDCl}_3$  solution,  $^1\text{H}$  NMR spectroscopy showed that approximately 38% of the  $\beta$ -ketoacid existed in the enol form **7.9b**.

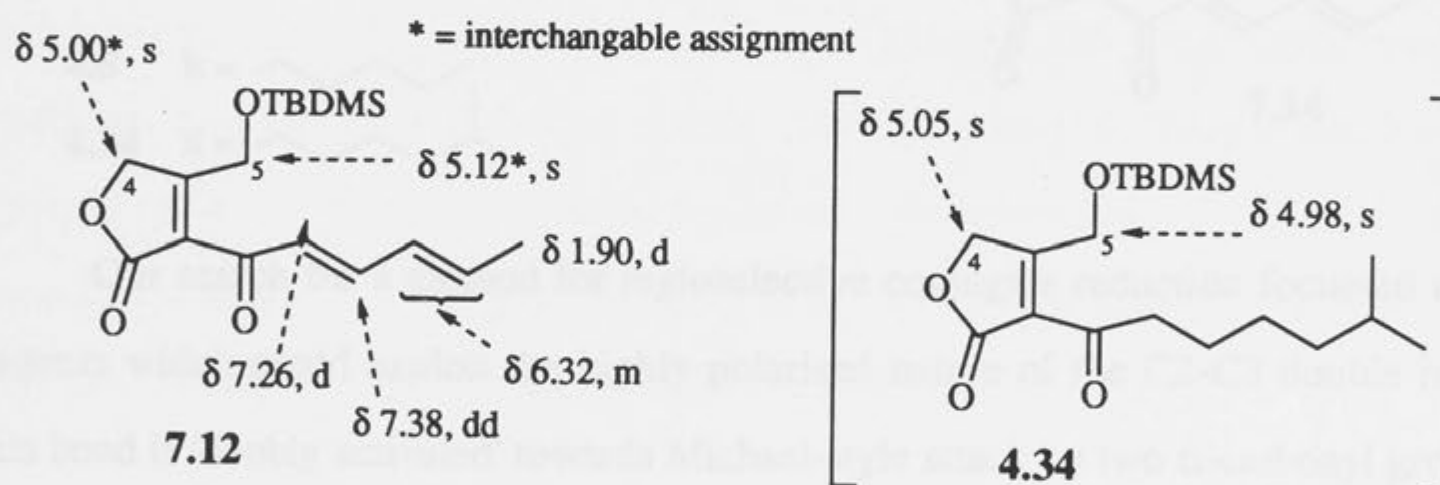


Scheme 7.3

Esterification with *t*-butyldimethylsilyl (TBDMS) protected dihydroxyacetone **4.7** using DCC / DMAP as described previously, gave the diketo dienyl ester **7.11** as the major product in the reaction mixture (Scheme 7.3). Assignment of the  $^1\text{H}$  NMR spectrum of the crude product was complicated by the presence of 50% of the enol form **7.11b** but all of the expected resonances were observed. The addition of an internal integration standard (triphenylmethane) allowed the yield of the cyclisation precursor **7.11** to be estimated as 55%. In retrospect, the ester **7.11** could probably be synthesised

more simply and in higher yield *via* Meldrum's acid, using the attractive route which we later briefly evaluated for the synthesis of 11-demethyl A-factor (**4.27**, see Section 4.4).

Cyclisation of the crude dienyl  $\beta$ -ketoester **7.11** was easily accomplished using radial chromatography on silica gel (10-30% EtOAc in hexane), a transformation directly analogous to that utilised in the synthesis of A-factor. The dienyl butenolide **7.12** was collected as an orange oil which could be crystallised from pentane solution as short orange needles. Successful cyclisation was confirmed by the appearance of two distinctive singlet resonances near  $\delta$  5.0 in the  $^1\text{H}$  NMR spectrum which closely resemble the H4 and H5 resonances of the butenolide intermediate **4.34** from the synthesis of A-factor (see below). The diene moiety was visible as four downfield proton resonances. HRMS established the molecular weight as 322 and the formula as expected ( $\text{C}_{17}\text{H}_{26}\text{O}_4\text{Si}$ ).



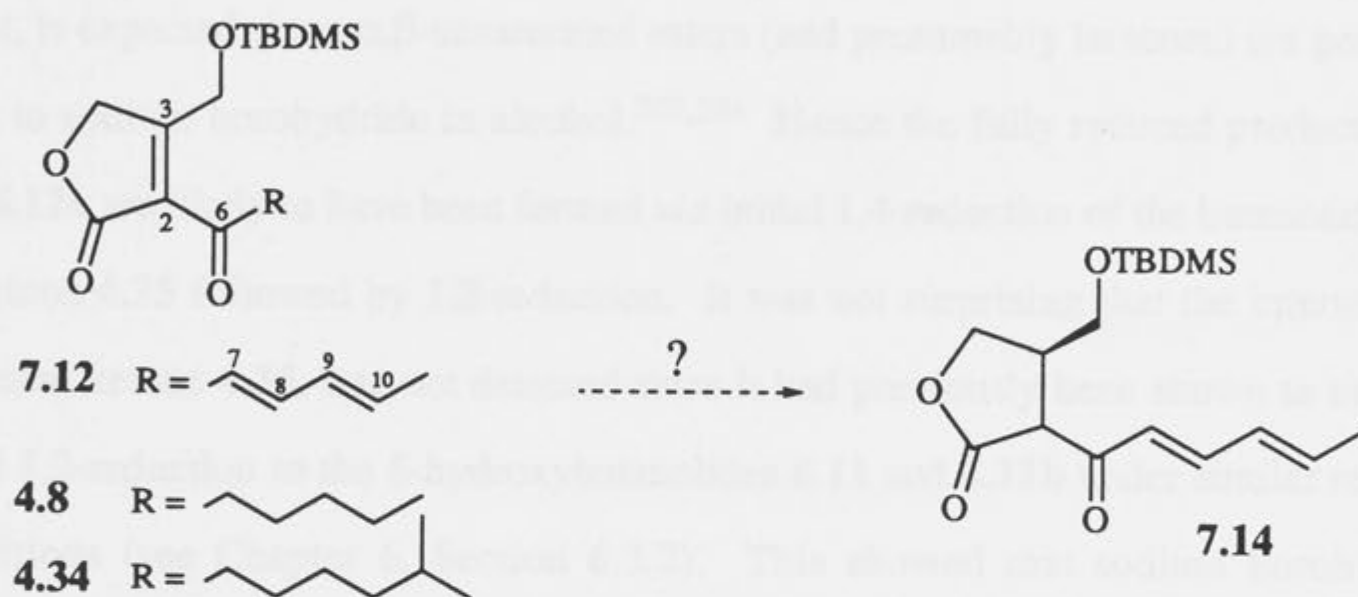
The combined yield for the esterification and cyclisation steps (34%) was lower than that attained in the synthesis of A-factor (65%) which may in part be due to an increased tendency of the butenolide to degrade in contact with silica gel.

Cyclisation was also catalysed by sodium hydride and diisopropylamine (as observed in the synthesis of A-factor), but the high levels of complex by-products observed precluded the synthetic use of these methods. The increased sensitivity of this butenolide intermediate **7.12** relative to the previously encountered butenolide **4.34** reflects the increased functionality present, and hence a method for its rapid conversion to the more stable saturated lactone **7.14** was required.



## 7.4 REGIOSELECTIVE REDUCTION OF THE DIENYL BUTENOLIDE 7.12

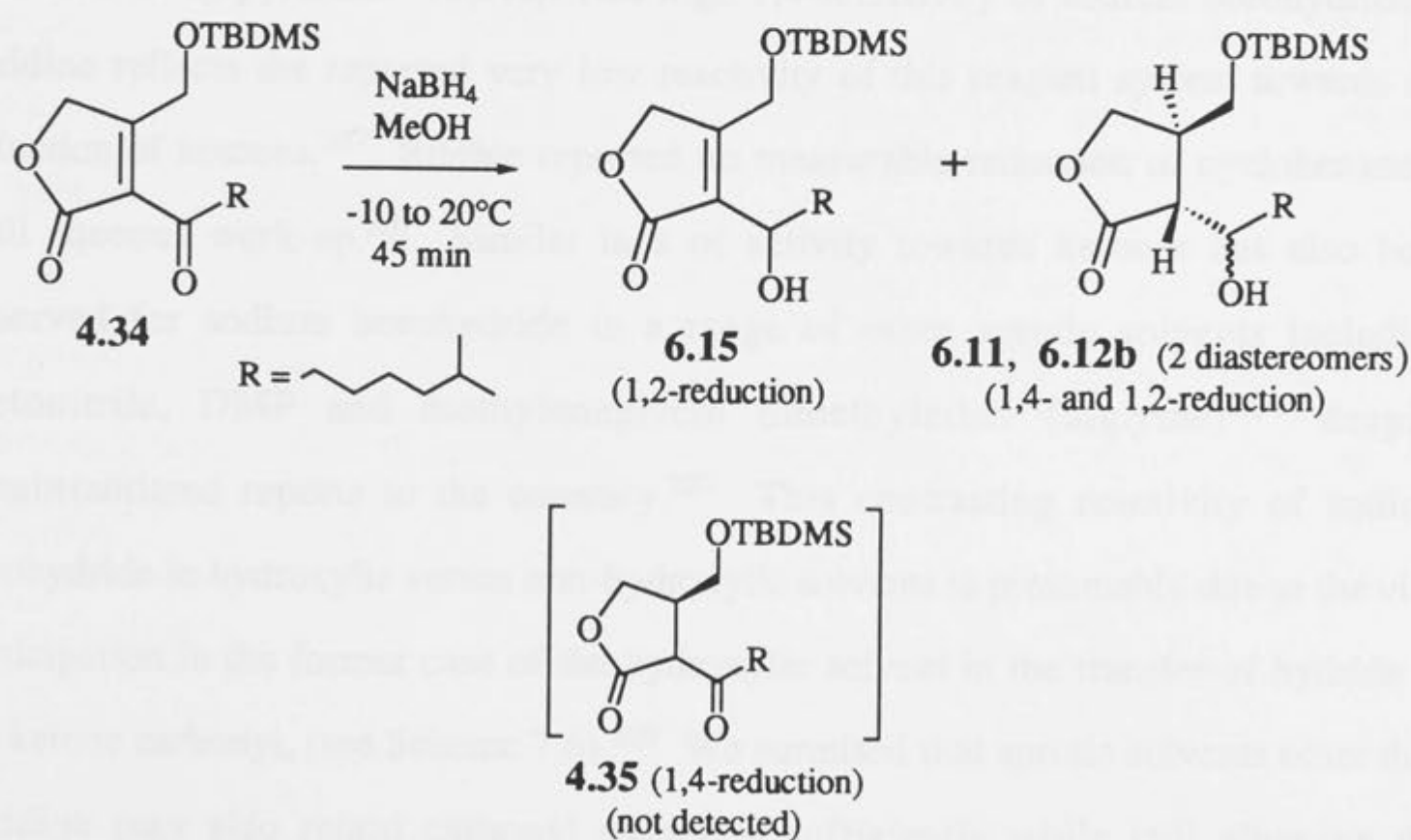
From this point the synthetic route diverges from that used for A-factor since catalytic hydrogenation, utilised for reduction of the butenolides **4.8** and **4.34**, is clearly unsuitable for the reduction of **7.12** due to the presence of the diene moiety.



Our search for a method for regioselective conjugate reduction focussed upon reagents which could exploit the highly polarised nature of the C2-C3 double bond. This bond is 'doubly activated' towards Michael-style attack by two  $\alpha$ -carbonyl groups. Thus we required a nucleophilic hydride reducing agent which would preferentially reduce a doubly activated olefin while leaving the mono-activated diene, the lactone and the ketone functionality unaffected.

A survey of the available reagents for conjugate reduction<sup>198,199</sup> revealed most to be either insufficiently selective, synthetically impractical or not readily available. One recently reported commercially available copper hydride reagent,<sup>200</sup>  $[(\text{Ph}_3\text{P})\text{CuH}]_6$ , held great promise due to its remarkable selectivity towards conjugate reduction. No 1,2-reduction is observed with  $\alpha,\beta$ -unsaturated esters, ketones or even aldehydes.<sup>201</sup> However, when illogical transport regulations covering the air-freighting of even small quantities of hydride reagents prevented its procurement, and time prevented its preparation, we re-examined some of the more readily available reagents.

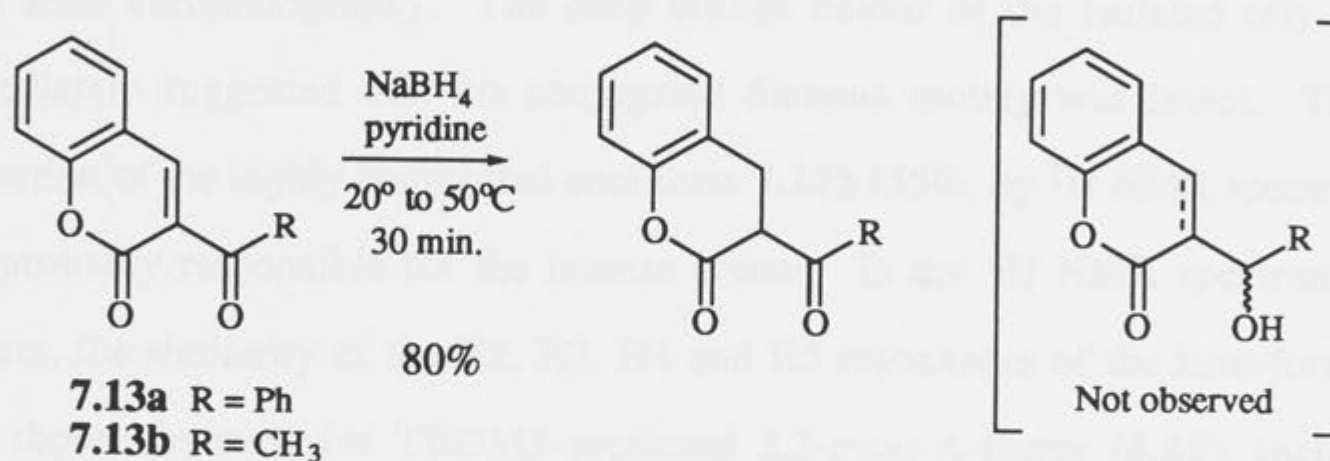
Sodium borohydride in alcohol usually converts simple  $\alpha,\beta$ -unsaturated ketones to mixtures consisting predominantly of the allylic alcohol (1,2-reduction) with varying smaller amounts of the saturated alcohol (from both 1,4- and ketone reduction).<sup>202</sup> As a model, the available butenolide **4.34** was treated with sodium borohydride (*ca* 1.5 fold excess over the amount required for full reduction) in methanol to give a ratio of 1,2- to 1,4- plus 1,2-reduction products (**6.15** : **6.11**+**6.12b**) of *ca* 1:20 (4% : 73% isolated) (Scheme 7.4). The detection of a small amount of **6.15**, despite the excess of reducing agent, is expected since  $\alpha,\beta$ -unsaturated esters (and presumably lactones) are generally inert to sodium borohydride in alcohol.<sup>203,204</sup> Hence the fully reduced products **6.11** and **6.12b** are likely to have been formed *via* initial 1,4-reduction of the butenolide **4.34** to ketone **4.35** followed by 1,2-reduction. It was not surprising that the intermediate saturated ketone **4.35**, was not detected since it had previously been shown to undergo rapid 1,2-reduction to the 6-hydroxybutanolides **6.11** and **6.12b** under similar reaction conditions (see Chapter 6, Section 6.3.2). This showed that sodium borohydride appeared to have a distinct preference for initial 1,4- rather than 1,2-reduction of the butenolide **4.34** and that it might be possible to isolate the desired saturated ketone **4.35** if reactivity towards its ketone moiety could be minimised.



Scheme 7.4



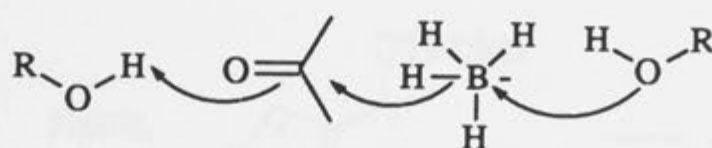
An example of the required selectivity was reported by Kadin<sup>205</sup> who demonstrated that the ketone and lactone activated olefin moiety of 3-benzoylcoumarin (**7.13a**) could be 1,4-reduced without any appreciable concomitant reduction of the ketone, using sodium borohydride in pyridine (Scheme 7.5). The subsequent extension of these conditions to the 3-acetylcoumarin **7.13b**<sup>206</sup> showed that the phenyl group was not essential for this selective reduction.



**Scheme 7.5 Literature Examples of Selective 1,4-Reduction**

Unfortunately, in our case these conditions were unsuitable since the dienyl butenolide **7.12** was found to be unstable in pyridine solution. <sup>1</sup>H NMR spectroscopy revealed 50% decomposition to a highly coloured unidentified mixture after less than 5 minutes in d<sub>5</sub>-pyridine. The reported high 1,4-selectivity of sodium borohydride in pyridine reflects the reported very low reactivity of this reagent system towards the reduction of ketones.<sup>207</sup> Ritchie reported no measurable reduction of cyclohexanone until aqueous work-up.<sup>207</sup> Similar lack of activity towards ketones has also been observed for sodium borohydride in a range of other aprotic solvents including acetonitrile, DMF and diethyleneglycol dimethylether (diglyme)<sup>208</sup> despite unsubstantiated reports to the contrary.<sup>203</sup> This contrasting reactivity of sodium borohydride in hydroxylic versus non-hydroxylic solvents is presumably due to the vital participation in the former case of the hydroxylic solvent in the transfer of hydride to the ketone carbonyl, (see Scheme 7.6).<sup>209</sup> We surmised that aprotic solvents other than pyridine may also retard carbonyl reduction sufficiently while still allowing the conjugate hydride addition to the doubly activated C3 position of our butenolide intermediate **7.12**.



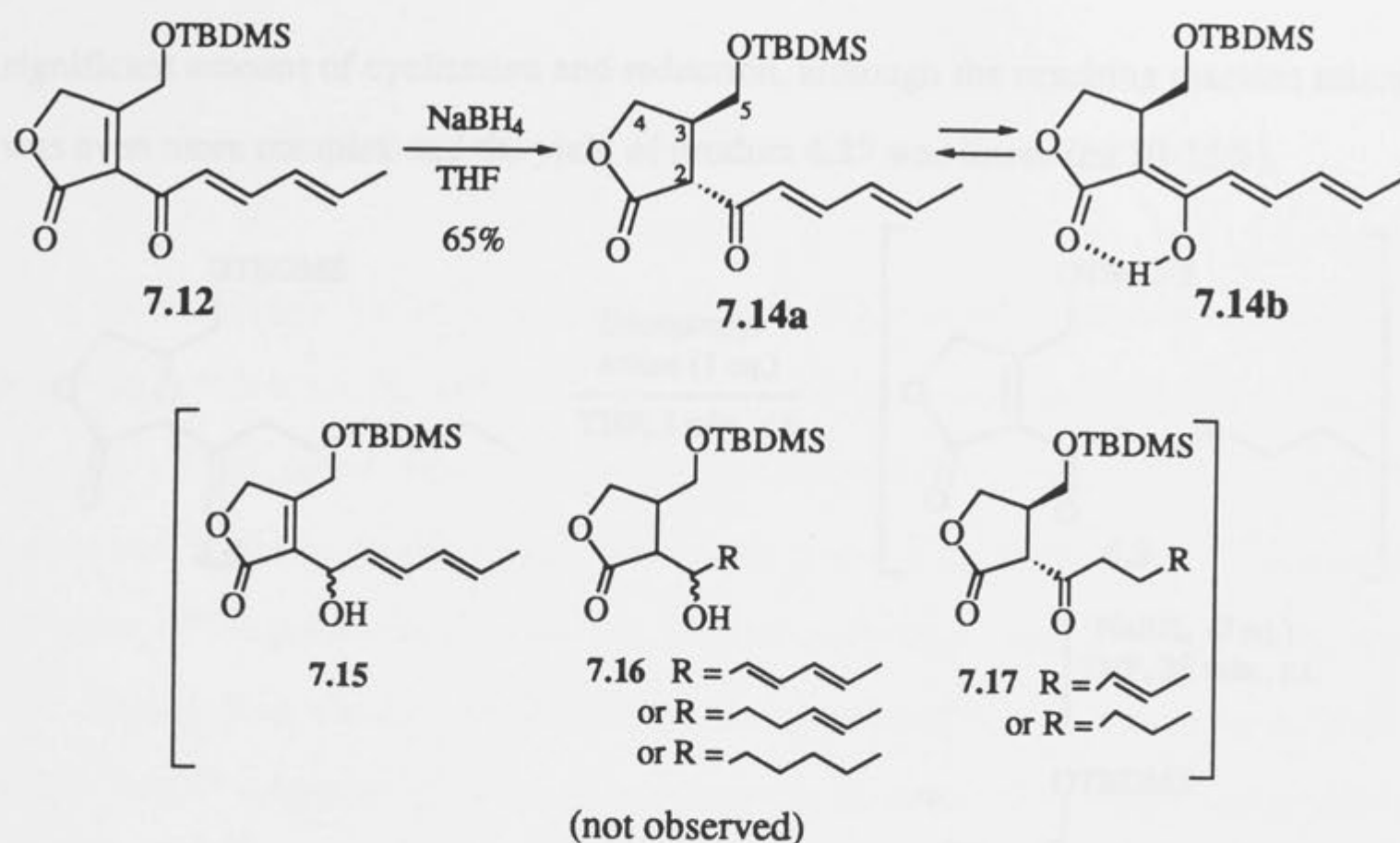


### Scheme 7.6 Participation of Hydroxylic Solvent in Reductions with Borohydride

Reduction of the dienyl butenolide **7.12** with an excess of sodium borohydride suspended in anhydrous THF (30 min, r.t.) gave, after rapid quenching with dilute aqueous hydrochloric acid, exclusive 1,4-reduction to form the butenolide **7.14** in 65% yield after chromatography. The deep orange colour of the isolated oily product immediately suggested that the conjugated dienone moiety was intact. The high proportion of the highly conjugated enol form **7.14b** (55%, by  $^1\text{H}$  NMR spectroscopy) was probably responsible for the intense colour. In the  $^1\text{H}$  NMR spectrum of the product, the similarity of the H2, H3, H4 and H5 resonances of the keto-form **7.14a** with those observed for TBDMS-protected 2,3-*trans*-A-factor (**4.35**), including a similar  $J_{2,3}$  coupling constant (**7.14a**: 7.3 Hz *cf.* **4.35**: 6.8 Hz), led to a tentative assignment of 2,3-*trans* geometry as depicted in Scheme 7.7. The four diene protons of the keto form **7.14a** appeared between  $\delta$  6.2 and 7.4 as observed for the butenolide precursor **7.12**, while those of the enol form **7.14b** were at slightly higher field ( $\delta$  5.9-7.1). In its EIMS, the product displayed a small parent ion at  $m/z$  324 whose composition was shown by HRMS to be  $\text{C}_{17}\text{H}_{28}\text{O}_4\text{Si}$ , as required for **7.14**. The base peak at  $m/z$  95 presumably resulted from the stabilised dienyl acyl cation confirming that the diene moiety remained unreduced.

Neither 6-hydroxy products **7.15** or **7.16** nor products resulting from the reduction of the diene moiety **7.17** were seen in either the crude reaction mixture or any chromatographic fractions, confirming that the required regioselective reaction had been achieved.



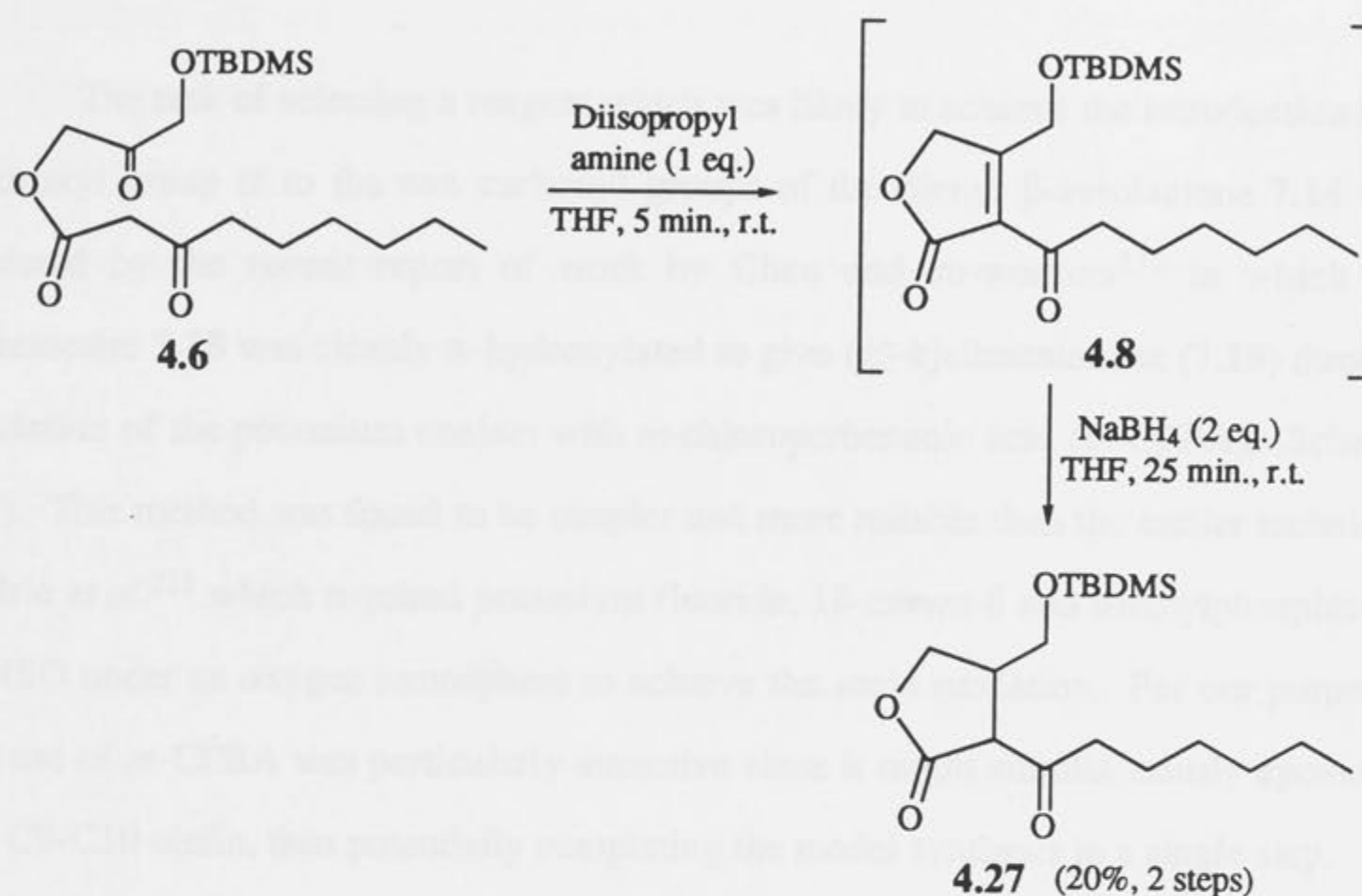


Scheme 7.7

The solubility of sodium borohydride in THF is low (*ca* 0.9 mg/mL)<sup>210</sup> but clearly sufficient to allow reduction. Any additional reducing agent remains suspended and presumably dissolves during the reaction to replace what has been used. Investigations were not carried out at this stage to test the scope and limitations of this convenient regioselective reaction, although it seems likely that similar reactivity may be achievable with other aprotic solvents, or with other selective borohydride reagents, such as sodium cyanoborohydride or quaternary ammonium borohydrides.<sup>210</sup>

The borohydride reduction also provides an alternative to catalytic hydrogenation for the reduction of the butenolide intermediates in the synthesis of A-factor (1.1) and its homologues. The use of THF makes the reduction step compatible with the base-catalysed cyclisation step, allowing the attractive possibility of achieving both in a 'one-pot' reaction (Scheme 7.8). Preliminary trials using the saturated diketoester 4.6 showed that cyclisation with one equivalent of diisopropylamine in THF (5 min, r.t.) followed by the addition of an excess of sodium borohydride (25 min, r.t.) gave a mixture of products from which a 20% yield of the acyl lactone 4.27 could be isolated (*cf.* 57% for the 2 step method, see Chapter 4). In the absence of the amine, sodium borohydride alone was sufficiently basic to promote a

significant amount of cyclisation and reduction, although the resulting reaction mixture was even more complex and the yield of product **4.27** was lower (*ca* 10-15%).



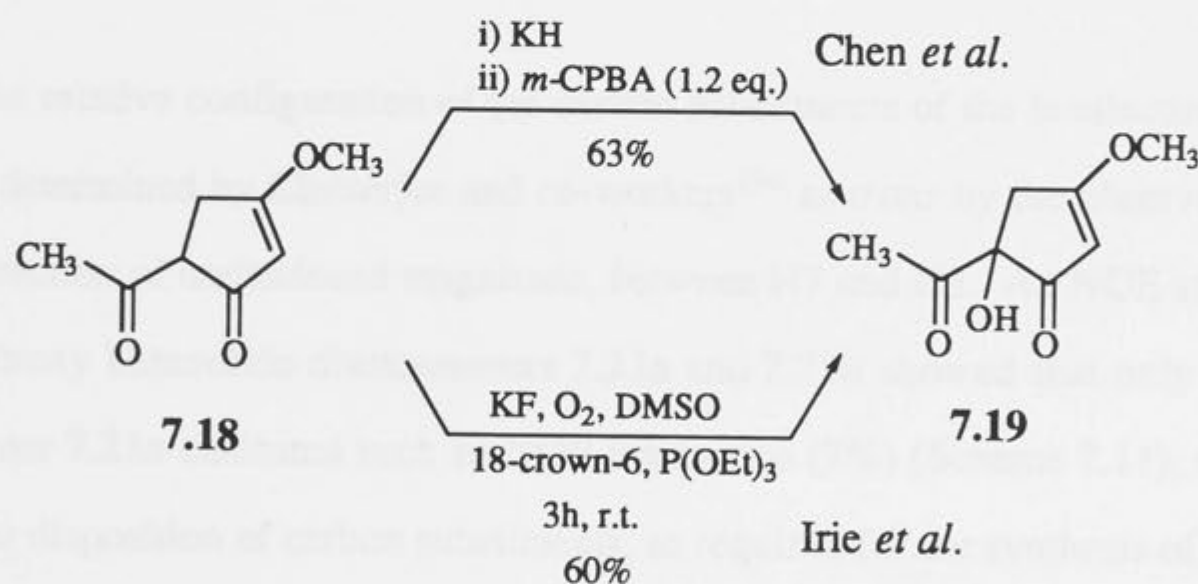
Scheme 7.8

This potentially useful synthetic short-cut was not pursued further but it raises the attractive possibility of extending the method with the use of chiral hydrides<sup>211</sup> to produce optically active autoregulators. A chiral reduction of this sort is likely to be directly analogous to the biosynthesis of optically active butanolides, where the chiral hydride donor would probably be nicotinamide-adenine dinucleotide phosphate, reduced form (NADPH).



### 7.5 $\alpha$ -HYDROXYLATION OF THE $\beta$ -KETOLACTONE 7.14

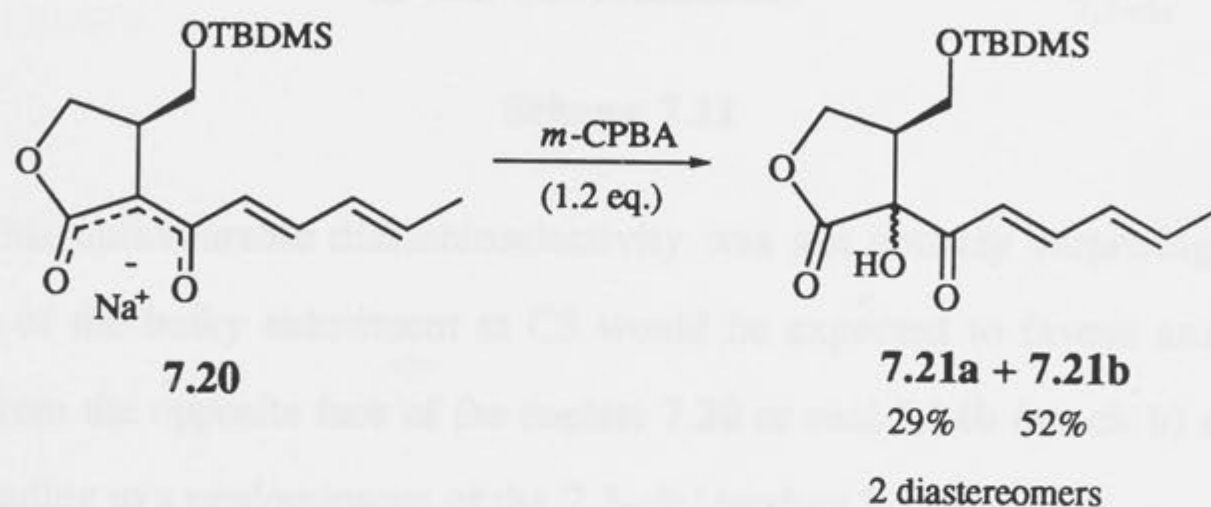
The task of selecting a reagent which was likely to achieve the introduction of a hydroxyl group  $\alpha$  to the two carbonyl groups of the dienyl  $\beta$ -ketolactone **7.14** was assisted by the recent report of work by Chen and co-workers<sup>212</sup> in which the  $\beta$ -ketoester **7.18** was cleanly  $\alpha$ -hydroxylated to give ( $\pm$ )-kjellmanianone (**7.19**) through oxidation of the potassium enolate with *m*-chloroperbenzoic acid (*m*-CPBA), (Scheme 7.9). This method was found to be simpler and more reliable than the earlier technique of Irie *et al.*<sup>213</sup> which required potassium fluoride, 18-crown-6 and triethylphosphite in DMSO under an oxygen atmosphere to achieve the same oxidation. For our purposes the use of *m*-CPBA was particularly attractive since it might simultaneously epoxidise the C9-C10 olefin, thus potentially completing the model synthesis in a single step.



Scheme 7.9 Synthesis of ( $\pm$ )-Kjellmanianone (**7.19**)

Thus the sodium enolate **7.20** of the  $\beta$ -keto lactone **7.14** was formed in THF using sodium hydride (1.1 eq.), before *m*-CPBA (1.2 eq.) was added with ultrasonication to assist dissolution. The initially yellow solution decolourised immediately suggesting that neither the highly conjugated enolate **7.20** nor any enol forms were present. Workup after 10 minutes gave a crude mixture which contained two products. After chromatographic separation, <sup>1</sup>H NMR analysis of the two colourless oils (29% and 52% yields) revealed them to be closely related. Both had

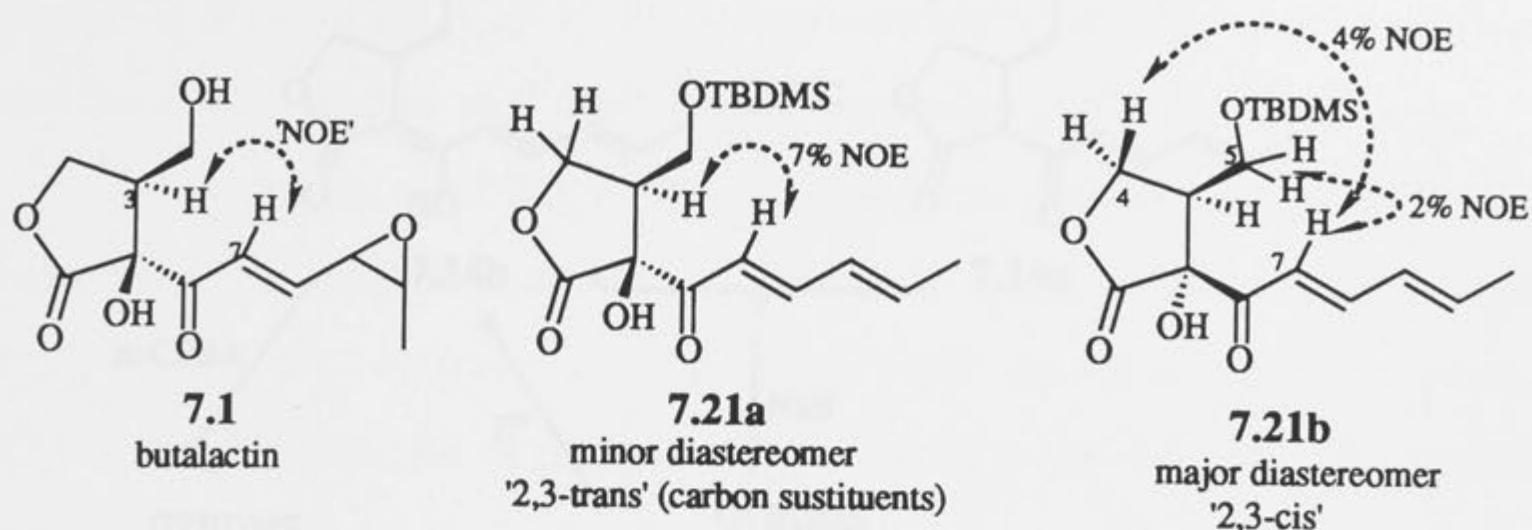
intact diene moieties, as shown by four olefinic resonances and both showed the familiar H3, H4 and H5 resonances of a saturated  $\gamma$ -lactone. Their identity as the two diastereomers of the required 2-hydroxy butanolides (Scheme 7.10) was supported by the lack of H2 doublets and the appearance of sharp hydroxyl singlets near  $\delta$  4.2. When EIMS and  $^{13}\text{C}$  NMR spectroscopy supported this identification it only remained to determine the relative stereochemistry of the new 2-hydroxyl substituents.



Scheme 7.10

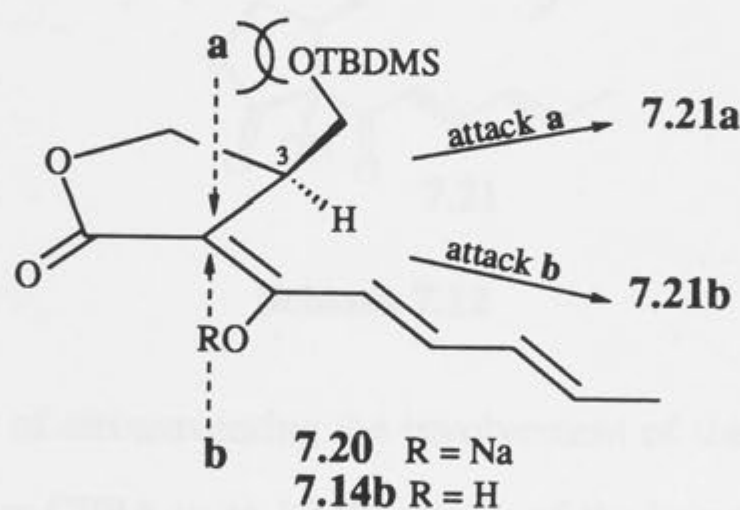
The relative configuration of the carbon substituents of the butalactin ring (7.1) had been determined by Chatterjee and co-workers<sup>194</sup> as *trans* by the observation of an NOE interaction of undisclosed magnitude, between H7 and H3. An NOE study of the two 2-hydroxy butanolide diastereomers 7.21a and 7.21b showed that only the *minor* diastereomer 7.21a exhibited such an NOE interaction (7%) (Scheme 7.11), suggesting a 2,3-*trans* disposition of carbon substituents, as required for the synthesis of butalactin (7.1). The major diastereomer 7.21b showed small NOE enhancements at H5 (2%) and at H4b (4%) upon irradiation of H7, indicating 2,3-*cis* relative geometry of the carbon substituents.



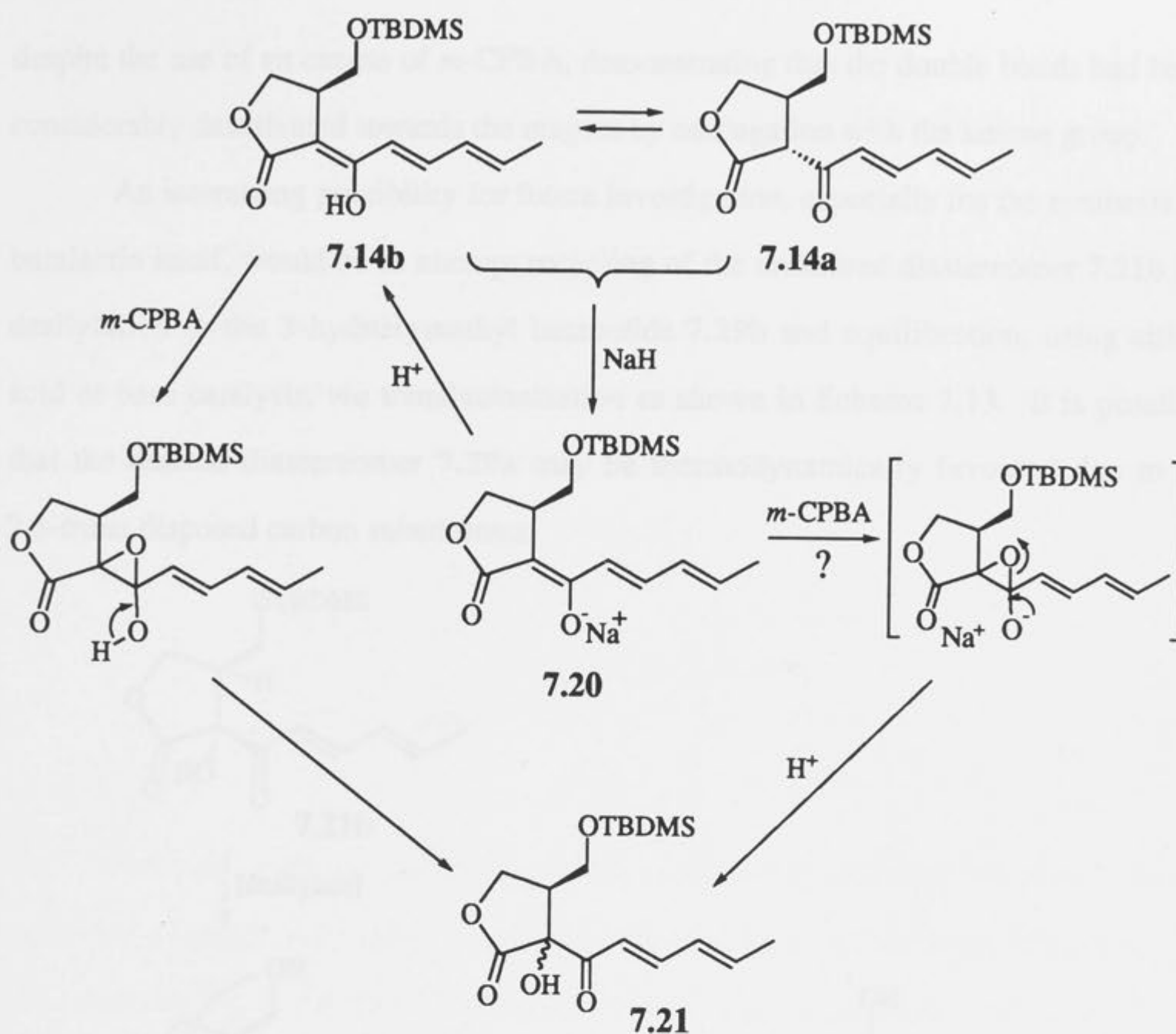


Scheme 7.11

This unfavourable diastereoselectivity was not entirely surprising since the presence of the bulky substituent at C3 would be expected to favour attack by the peracid from the opposite face of the enolate **7.20** or enol **7.14b** (attack **b**) as depicted below, leading to a predominance of the '2,3-cis' product **7.21b**.



It appeared likely to us that the species being attacked by the *m*-CPBA was the enol **7.14b** rather than the enolate **7.20** as implied by Chen *et al.*,<sup>212</sup> since the latter would be expected to be protonated by the peroxyacid or the 10-20% *m*-chlorobenzoic acid which is present in commercial *m*-CPBA. As discussed in Chapter 4, the protonation of  $\beta$ -dicarbonyl enolates occurs on oxygen<sup>158</sup>, initially forming the enol **7.14b** which could then undergo epoxidation and rearrangement to the 2-hydroxybutanolides **7.21** as shown in Scheme 7.12.



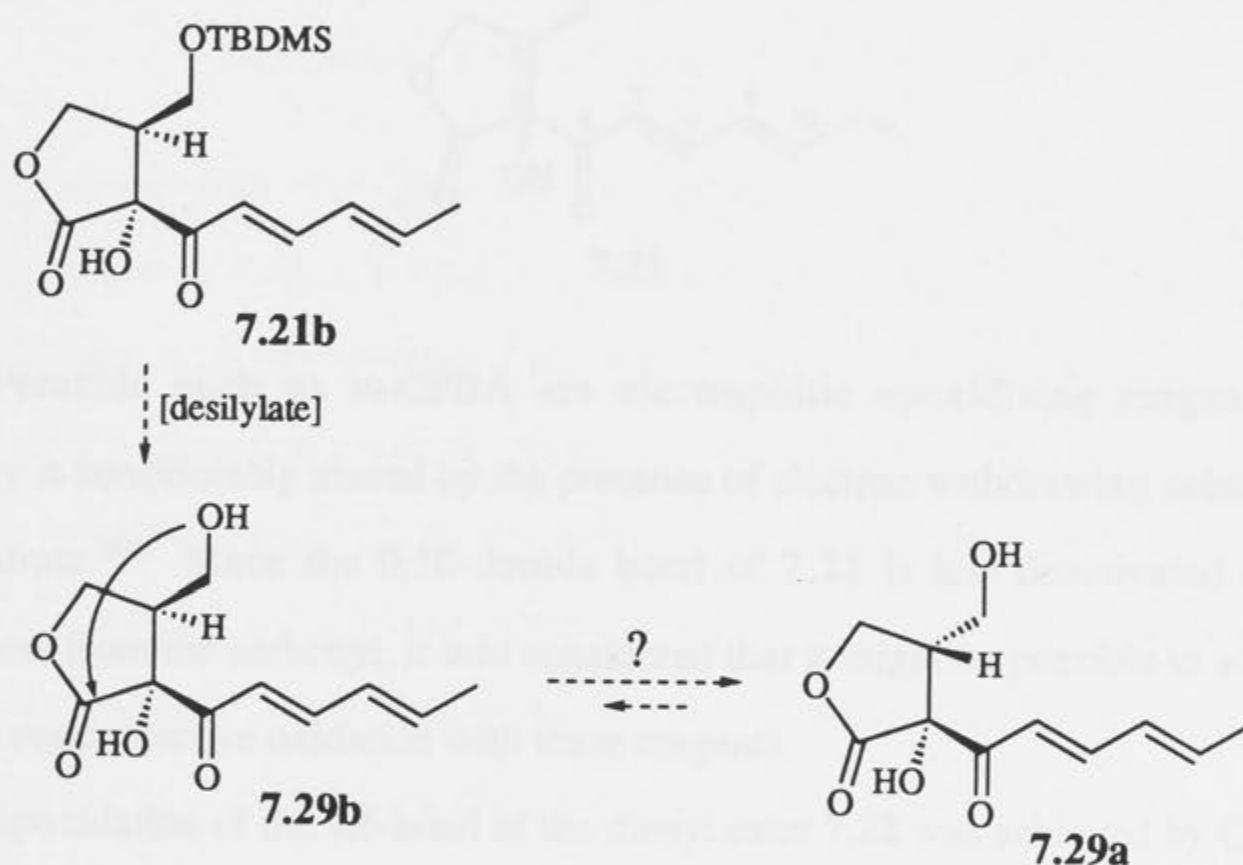
Scheme 7.12

The possibility of circumventing the involvement of the enolate was tested by the addition of excess *m*-CPBA to an NMR sample of the butanolide (7.14) in CDCl<sub>3</sub>. Within 10 minutes the enol 7.14b, which represents 55% of the sample, was fully converted into a similar mixture of the previously identified 2-hydroxy butanolides 7.21a and 7.21b, whereas the keto form 7.14a remained largely unchanged even after 1 hour. This test confirmed that the enol form 7.14b is rapidly oxidised but the sluggishness of the keto-enol conversion prevents the oxidation going to completion in a reasonable period. It appears that formation of the enolate before *m*-CPBA oxidation probably serves only to allow full conversion of the starting material to the enol form 7.14b prior to oxidation, although some direct oxidation of the enolate 7.20 (Scheme 7.12) cannot be ruled out. No evidence for epoxides was noted in this reaction



despite the use of an excess of *m*-CPBA, demonstrating that the double bonds had been considerably deactivated towards the reagent by conjugation with the ketone group.

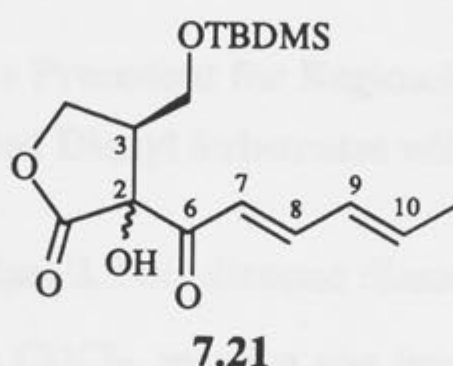
An interesting possibility for future investigation, especially for the synthesis of butalactin itself, would be to attempt recycling of the undesired diastereomer **7.21b** by desilylation to the 3-hydroxymethyl butanolide **7.29b** and equilibration, using either acid or base catalysis, *via* translactonisation as shown in Scheme 7.13. It is possible that the desired diastereomer **7.29a** may be thermodynamically favoured due to its 2,3-*trans* disposed carbon substituents.



Scheme 7.13

## 7.6 EPOXIDATION OF THE DIENONE MOIETY

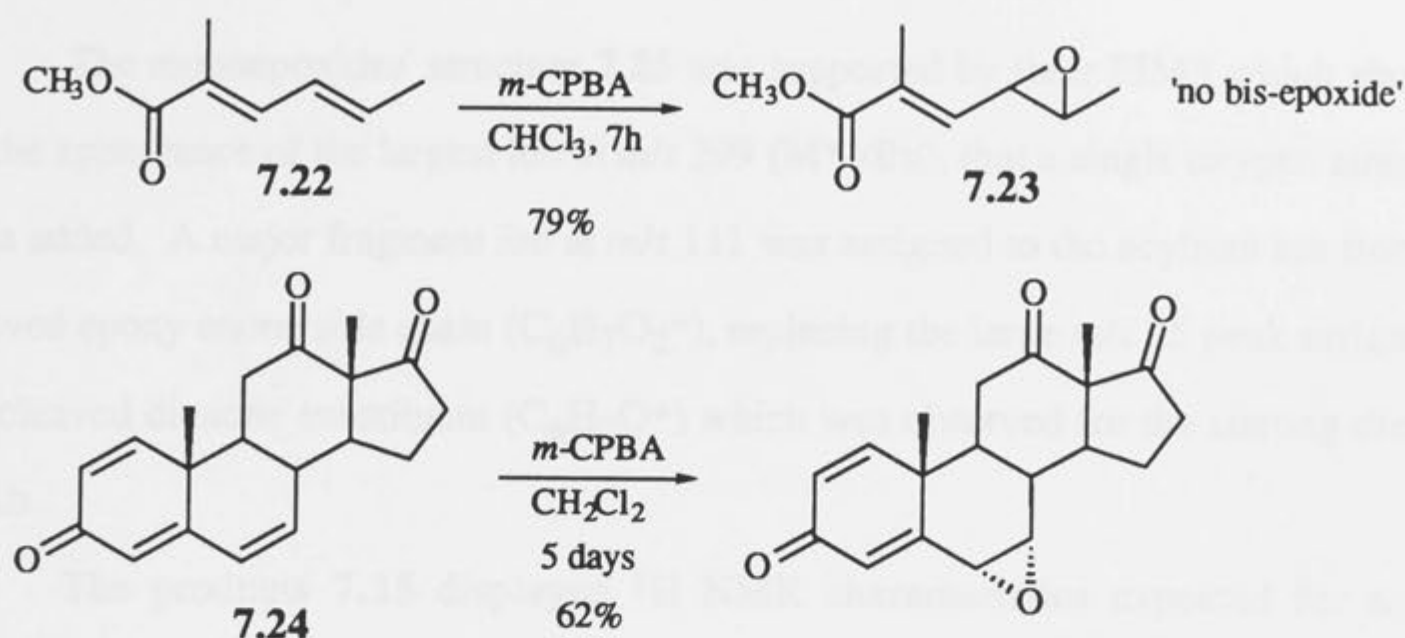
The electron deficient double bond of  $\alpha,\beta$ -unsaturated ketones can often be epoxidised using a nucleophilic reagent such as the sodium salt of *t*-butyl hydroperoxide or related reagents,<sup>214</sup> but for dienones such as **7.21**, the more electron deficient 7,8-double bond may be preferentially oxidised.



Peracids such as *m*-CPBA are electrophilic epoxidising reagents whose reactivity is considerably abated by the presence of electron withdrawing substituents in the substrate.<sup>106</sup> Since the 9,10-double bond of **7.21** is less deactivated due to its remoteness from the carbonyl, it was considered that it might be possible to achieve the required regioselective oxidation with these reagents.

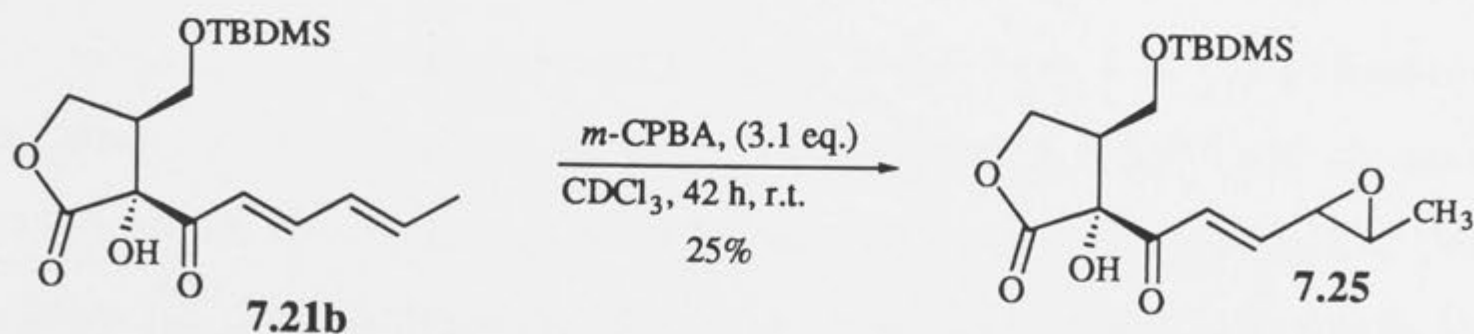
Epoxidation of the  $\gamma,\delta$ -bond of the dienyl ester **7.22** was achieved by Ceroni and Sequin (Scheme 7.14) using *m*-CPBA in  $\text{CHCl}_3$ .<sup>215</sup> For dienyl ketones, however, the reaction is more challenging due to both the stronger deactivating influence of the ketone and to the sensitivity of ketones to Baeyer-Villiger oxidation.<sup>216</sup> The regioselective oxidation of **7.24**<sup>217</sup> (Scheme 7.14) provides a rare but encouraging example of the required selective epoxidation, although a very long reaction time (5 days) was required. Both these examples show that the distal  $\gamma,\delta$ -double bond of the dienone system can be preferentially epoxidised even when the  $\alpha,\beta$ -double bond is more substituted, a feature which promotes epoxidation by peracids.





**Scheme 7.14 Literature Precedent for Regioselective  $\gamma,\delta$ -Epoxidation of Deactivated Dienyl Substrates with *m*-CPBA**

When the more abundant 2,3-*cis* dienone diastereomer **7.21b** was treated with 1.1 equivalents of *m*-CPBA in  $\text{CDCl}_3$ , reaction was found to be very slow. Monitoring by  $^1\text{H}$  NMR spectroscopy revealed that approximately 30% of the starting material had reacted after 16 hours at room temperature, forming one major and several minor products, as judged by comparative integration of the emerging new methyl doublets between  $\delta$  1.2 and 1.8 and the diminishing methyl doublet of the starting dienone at  $\delta$  1.9. After the addition of a further quantity of *m*-CPBA (2.0 eq.) followed by 26 more hours standing at room temperature only approximately 10% of the starting material remained and the now rather complex reaction mixture was worked up. The major products **7.25** were isolated as an inseparable 1:1 diastereomeric mixture after preparative TLC in 25% yield (Scheme 7.15), in addition to some starting material (*ca.* 8%) and a complex mixture of unidentified polar by-products.



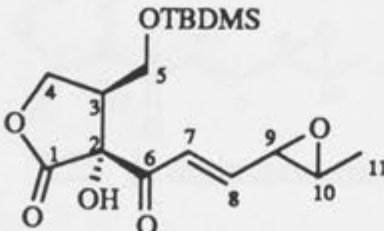
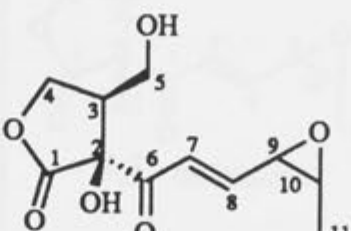
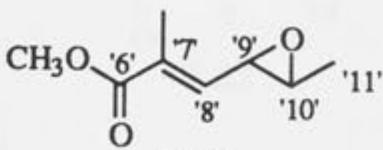
**Scheme 7.15**

The monoepoxides' structure **7.25** was supported by their EIMS which showed, by the appearance of the largest ion at  $m/z$  299 ( $M^+ - tBu$ ), that a single oxygen atom had been added. A major fragment ion at  $m/z$  111 was assigned to the acylium ion from the cleaved epoxy enone side chain ( $C_6H_7O_2^+$ ), replacing the large  $m/z$  95 peak assigned to the cleaved dienone substituent ( $C_6H_7O^+$ ) which was observed for the starting dienone **7.21b**.

The products **7.25** displayed  $^1H$  NMR characteristics expected for a 1 : 1 diastereomeric mixture of the 9,10-mono epoxides **7.25**, as demonstrated by comparison with  $^1H$  NMR data for butalactin (**7.1**)<sup>194</sup> and *cis*-epoxide **7.23**<sup>215</sup> (Table 7.1). Most of the resonances of the two diastereomers were superimposed or so close in chemical shift that, with the exception of the H4 resonances, it was not possible to extract individual coupling data. The doublet signal representing both of the terminal methyl groups was shifted upfield to  $\delta$  1.40 from the observed position of  $\delta$  1.90 in the starting dienone, as expected for a methyl substituent of a *cis*-epoxide (*cf.* **7.23**, Table 7.1). The two epoxide protons (H9 and H10) of the diastereomers appeared superimposed as multiplets at  $\delta$  3.22 and 3.0, respectively, positions also consistent with the *cis*-epoxide structure **7.25** (*cf.* **7.23**). The protons of the unreacted olefin moieties (H7 and H8) were not resolved, appearing as a single four proton multiplet at  $\delta$  6.8, while the chemical shifts of the protons of the hydroxymethyl butyrolactone moieties (H3, H4 and H5) were similar to those of butalactin (**7.1**).



Table 7.1  $^1\text{H}$  NMR Comparison of Epoxides 7.25 with Literature Models

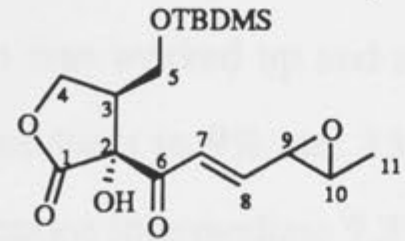
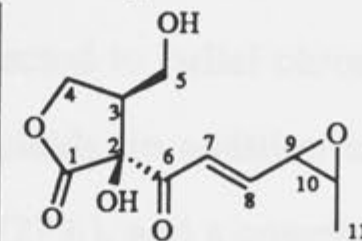
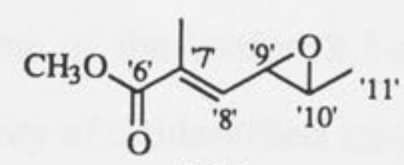
<div style="display: flex; justify-content: space-around; align-items: flex-end;"> <div style="text-align: center;">  <p><b>7.25</b> (2 diastereomers)</p> </div> <div style="text-align: center;">  <p><b>7.1</b> butalactin</p> </div> <div style="text-align: center;">  <p><b>7.23</b></p> </div> </div>			
Proton No. <sup>i</sup>	$\delta$ $^1\text{H}$ NMR	$\delta$ $^1\text{H}$ NMR	$\delta$ $^1\text{H}$ NMR
<b>H3</b> (CH)	3.0, m	2.95, m	ii
<b>H4</b> (CH <sub>2</sub> )	4.33, 2xdd	4.39, dd	ii
	4.52, 2x't'	4.57, dd	
<b>H5</b> (CH <sub>2</sub> )	3.75, 'd'	3.84, dd	ii
		3.89, dd	
<b>H7</b> (CH)	6.8, m	6.82, dd	ii
<b>H8</b> (CH)	6.8, m	7.06, dd	6.29, dq
<b>H9</b> (CH)	3.22, m	3.60, ddd	3.32, dd
<b>H10</b> (CH)	3.0, m	3.38, dq	3.03, dq
<b>H11</b> (CH <sub>3</sub> )	1.4, d	1.28, d	1.40, d

Notes i) protons numbered as shown above.

ii) equivalent proton not present.

The  $^{13}\text{C}$  NMR spectrum of the product **7.25** clearly showed its diastereomeric composition by the appearance of many of the resonances (especially those near the epoxide moiety) as 1:1 'doublets'. It was readily apparent that resonances due to the C9 olefin of the starting diene **7.21b** ( $\delta$  130.2 and 144.6) had been replaced by new peaks corresponding to a 9,10-epoxide moiety at  $\delta$  57 - 58 (see Table 7.2). Comparison of the  $^{13}\text{C}$  NMR data with those of literature models butalactin (**7.1**)<sup>194</sup> and *cis*-epoxide **7.23**<sup>215</sup>, reveals good correlation when structural differences are considered, as shown in Table 7.2. The monoepoxides **7.25** are protected diastereomers of butalactin (**1.1**) and their formation demonstrates that all of the functionality required for butalactin synthesis can be assembled by this route.

Table 7.2  $^{13}\text{C}$  NMR Comparison of Epoxides 7.25 with Literature Models

<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;">  <p><b>7.25</b> (2 diastereomers)</p> </div> <div style="text-align: center;">  <p><b>7.1</b> butalactin</p> </div> <div style="text-align: center;">  <p><b>7.23</b></p> </div> </div>			
Carbon No. <sup>i</sup>	$\delta$ $^{13}\text{C}$ NMR	$\delta$ $^{13}\text{C}$ NMR	$\delta$ $^{13}\text{C}$ NMR
C1 (C=O)	174.2 <sup>iii</sup>	174.1	ii)
C2 (quat. C)	82.2 <sup>iii</sup>	81.9	ii)
C3 (CH)	50.6 <sup>iii</sup>	43.9	ii)
C4 (CH <sub>2</sub> )	67.4 <sup>iii</sup>	69.0	ii)
C5 (CH <sub>2</sub> )	58.7, 58.8 <sup>iv</sup>	59.8	ii)
C6 (C=O)	194.1 <sup>iii</sup>	193.9	ii)
C7 (CH)	124.8, 125.0	125.4	132.1
C8 (CH)	146.6, 146.7	145.0	138.5
C9 (CH)	57.3, 57.4 <sup>iv</sup>	55.6	55.3
C10 (CH)	57.9, 58.1 <sup>iv</sup>	56.0	56.1
C11 (CH <sub>3</sub> )	17.5 <sup>iii</sup>	13.1	17.5

Notes i) carbons numbered as shown above.  
 ii) equivalent carbon not present.  
 iii) represents unresolved peaks.  
 iv) assignments may be interchanged.

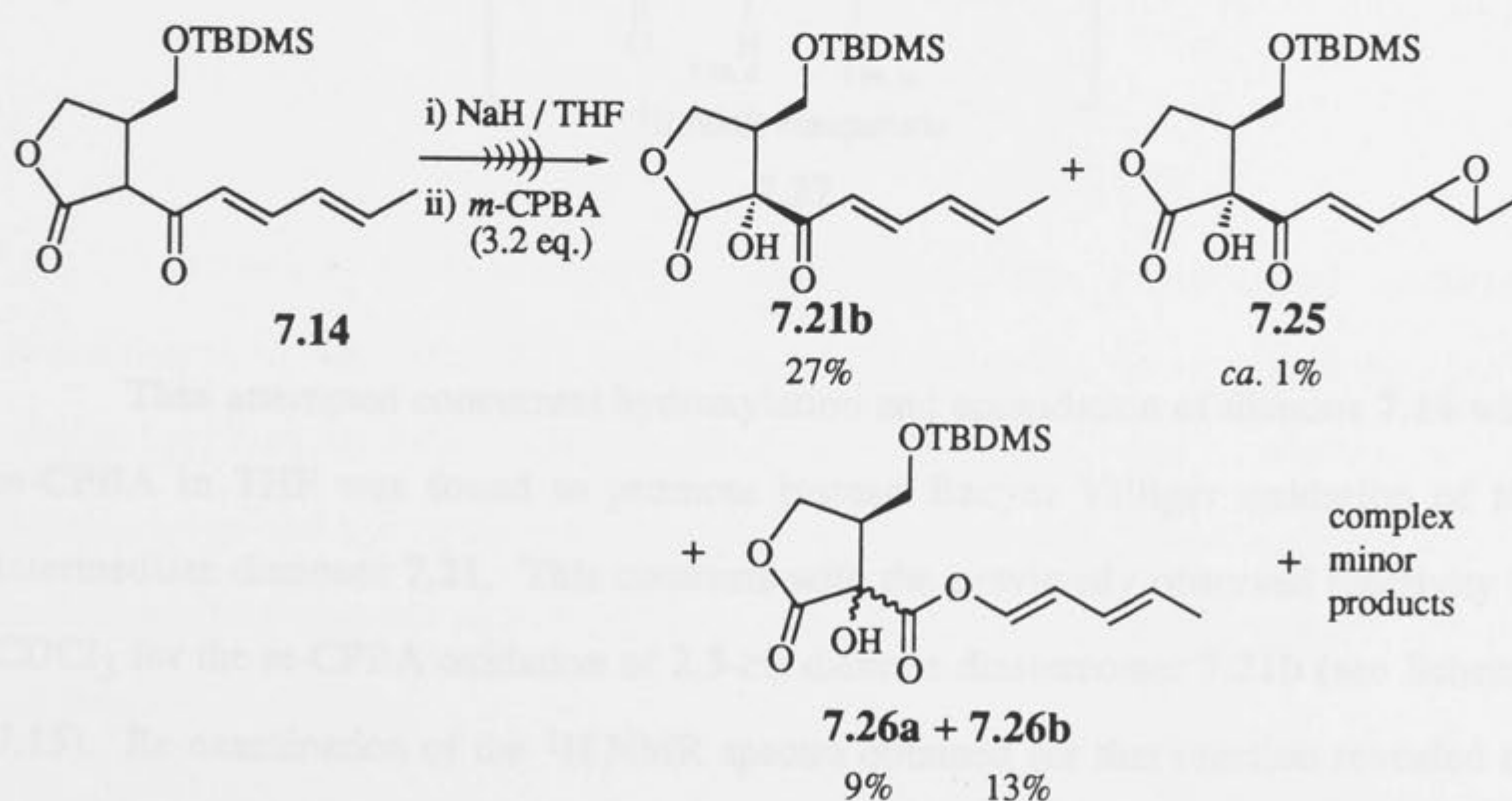
It was recognised that since the hydroxylation and epoxidation steps both utilise *m*-CPBA, they could potentially be combined, thereby eliminating the need to isolate the intermediate hydroxy dienones **7.21a** and **7.21b**.

Thus the sodium enolate of the dienone **7.14** in THF was treated with 3.2 equivalents of *m*-CPBA with ultrasonication to assist dissolution. Monitoring by TLC showed that formation of the expected 2-hydroxy dienone intermediates **7.21a** and **7.21b** was completed in less than 10 minutes but that further reaction was slower. A small amount of a component corresponding to the mono-epoxides **7.25** was noted during the first hour but this was subsequently overshadowed by two other products at higher  $R_f$ . After 16 hours, additional *m*-CPBA was added (1.0 eq.) since the hydroxy



dienone intermediates **7.21a** and **7.21b** had not been fully consumed. After 29 hours the reaction was worked up and subjected to radial chromatography to afford the two higher  $R_f$  products in 9% and 13% yields, in addition to some of the 'major' 2,3-*cis* hydroxy dienone intermediate **7.21b** (27%), and a complex array of unidentified minor products. None of the 'minor' 2,3-*trans* hydroxy dienone **7.21a** was detected and only a trace (*ca* 1%) of the mono-epoxides **7.25** was seen by NMR spectroscopy in the crude reaction mixture.

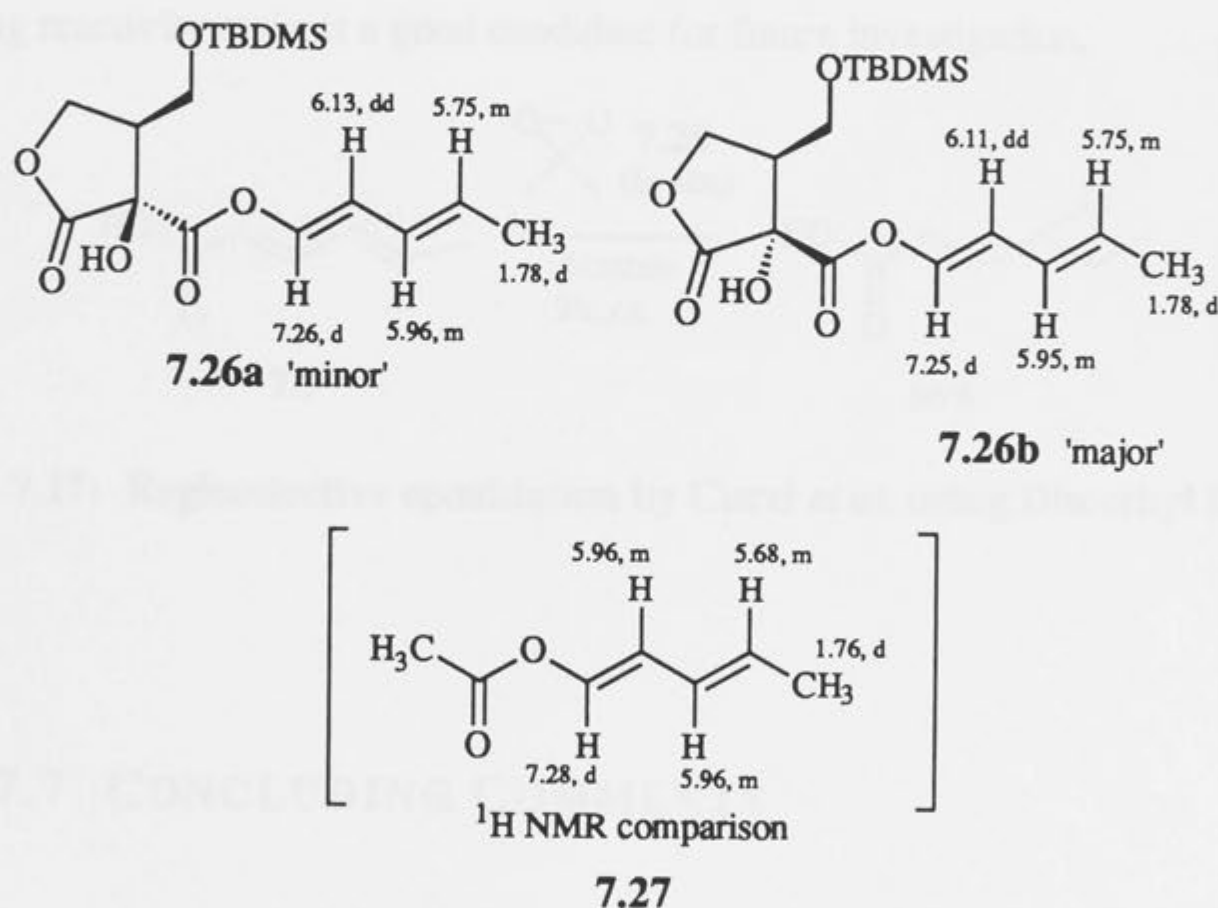
The two new products showed very similar EI mass spectra, both displaying ions at  $m/z$  356 ( $M^+$ ) and 299 ( $M^+ - C_4H_9$ ) which suggested that they were isomeric with the previously isolated 2-hydroxy mono-epoxides **7.25**. In contrast with the epoxides **7.25**, however, they did not exhibit a fragment ion at  $m/z$  111 corresponding to cleavage of an epoxidised dienone side chain.  $^1H$  NMR spectroscopy was more informative, showing that the new, closely related oxidation products both possessed four olefinic protons but no resonances attributable to epoxide protons ( $\delta$  3.0 - 3.3). Since both also displayed a singlet due to an hydroxyl proton near  $\delta$  3.7 and a set of resonances characteristic of an intact O-silylated hydroxymethyl butyrolactone moiety, the compounds were assigned as the diastereomers of the Baeyer Villiger oxidation product **7.26** (Scheme 7.16).



Scheme 7.16

(*E,E*)-1,3-pentadienyl acetate (**7.27**)<sup>218</sup> provided a good <sup>1</sup>H NMR model for the dienyl ester moiety, and the close correspondence of signals (see below) gave strong support for the proposed structures. It is somewhat surprising that these products were isolated unepoxidised since their double bonds were no longer deactivated by conjugation with the carbonyl group. It is probable that the complex array of minor by-products contains compounds resulting from their epoxidation.

The relative configuration of the diastereomeric Baeyer Villiger oxidation products **7.26a** and **7.26b** was not determined but it appears likely that the more abundant diastereomer **7.26b** shares the 2,3-*cis* configuration of the more abundant 2-hydroxy dienone intermediate **7.21b**, as depicted below.

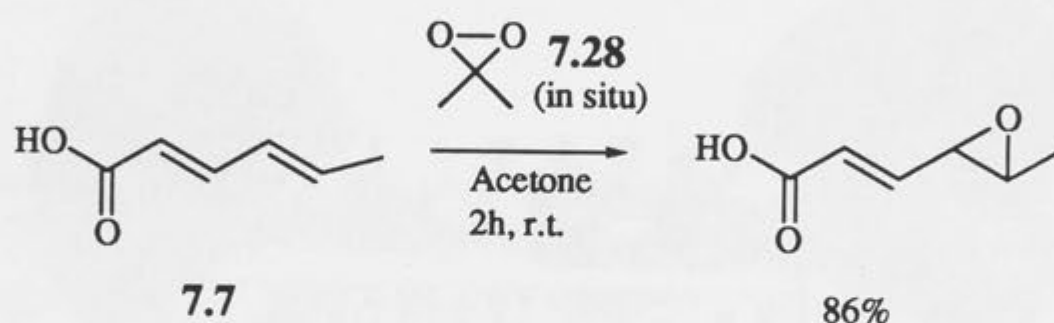


Thus attempted concurrent hydroxylation and epoxidation of dienone **7.14** with *m*-CPBA in THF was found to promote instead Baeyer Villiger oxidation of the intermediate dienones **7.21**. This contrasts with the previously observed reactivity in CDCl<sub>3</sub> for the *m*-CPBA oxidation of 2,3-*cis* dienone diastereomer **7.21b** (see Scheme 7.15). Re-examination of the <sup>1</sup>H NMR spectra obtained for that reaction revealed no sign of either of the Baeyer Villiger products (**7.26a** or **7.26b**), despite the long reaction time (42 h), and the excess of reagent employed (3.1 equiv.). The reaction solvent



appears to crucially affect the mode of oxidation by *m*-CPBA, and future work on optimisation of this epoxidation step will utilise  $\text{CHCl}_3$  or  $\text{CH}_2\text{Cl}_2$  rather than ethereal solvents.

Another promising approach to achieve regioselective epoxidation may be through the use of dimethyl dioxirane (**7.28**),<sup>219</sup> a powerful, recently developed epoxidising agent which is reported to cause no Baeyer Villiger oxidation during epoxidation of enones.<sup>220,221</sup> While its reactivity towards conjugated dienones has not been reported, it has been shown to preferentially epoxidise the distal 4,5-olefin of sorbic acid (**7.7**, Scheme 7.17)<sup>220</sup>, demonstrating its electrophilic nature. Although the reagent is inconvenient to prepare, especially on a large scale,<sup>222</sup> its ease of use and promising reactivity make it a good candidate for future investigation.



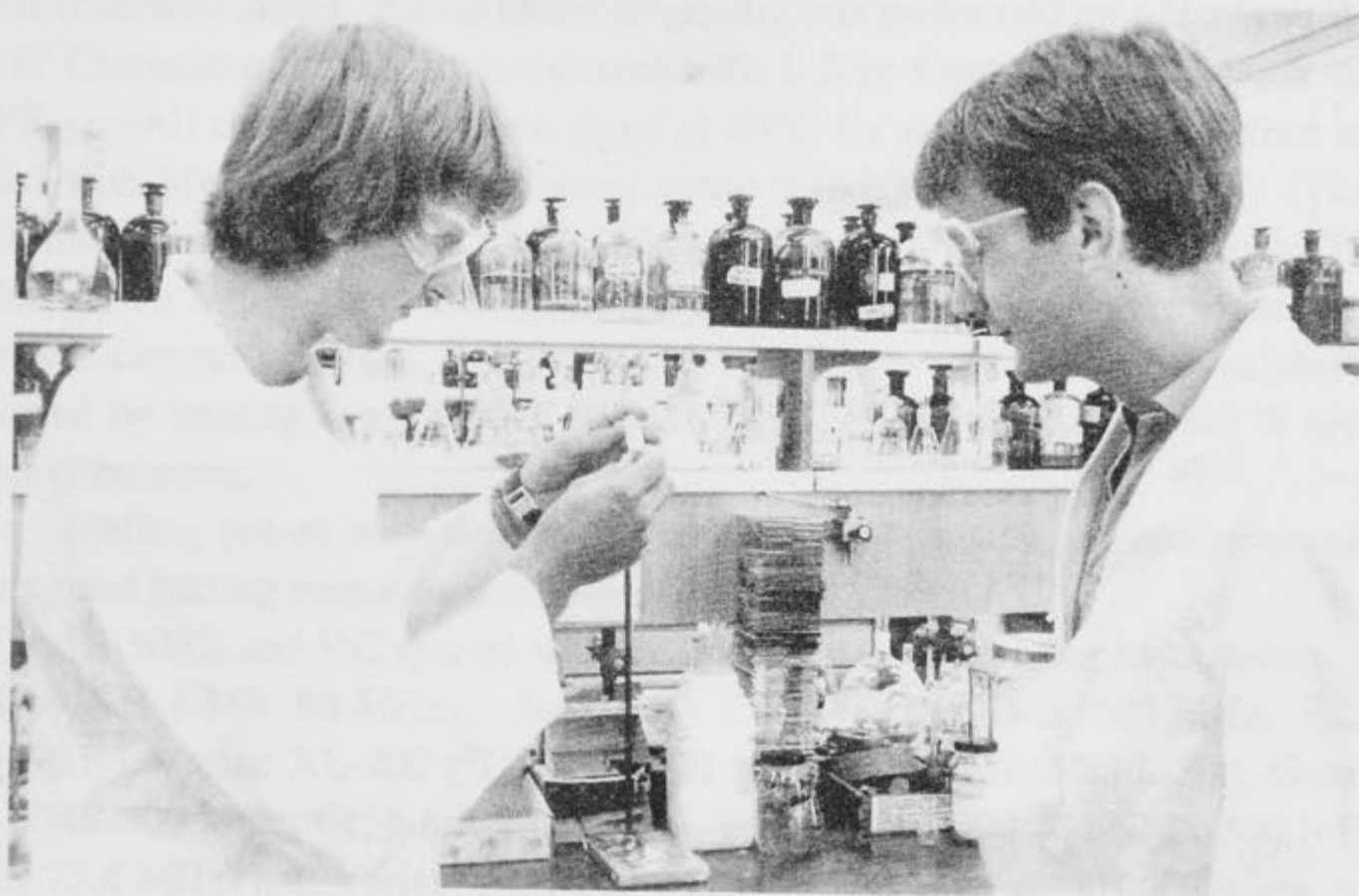
Scheme 7.17: Regioselective epoxidation by Curci *et al.* using Dimethyl Dioxirane

## 7.7 CONCLUDING COMMENTS

Synthesis of the diastereomeric monoepoxides **7.25**, which are silyl protected stereoisomers of butalactin (**7.1**), has demonstrated that our biomimetic approach can be used to install all the functionality required for the target molecule. Although the final hydroxylation and epoxidation steps require improvements in their diastereoselectivity and regioselectivity, the stage is set for application of this synthetic route to the first synthesis of butalactin.

## GENERAL PROCEDURES

## EXPERIMENTAL



**The author (left) and crystallographer Dr Jonathon White  
admiring crystals of A-factor hemiketal**



## GENERAL PROCEDURES

Solvents and reagents were purified, where necessary, according to the procedures described by Perrin and co-workers<sup>223</sup>. All organic extracts were dried over anhydrous magnesium sulphate. The bulk of the solvents were removed under reduced pressure using a rotary evaporator. Solvent traces were removed by evaporation under high vacuum (< 0.5 mm Hg). All air and moisture sensitive reactions were carried out under a positive pressure of anhydrous argon using flame-dried glassware which was cooled under anhydrous argon. Commercial *n*-butyllithium solutions (ca 1.6 M in hexane) were assayed by titration with 2,5-dimethoxybenzyl alcohol<sup>224</sup> as described by Winkle and co-workers.<sup>225</sup> Phosphate buffers (pH 3.5, 5.0, 7.0) were prepared by the addition of phosphoric acid (100%) to a 1 M aqueous solution of disodium hydrogen phosphate until the required pH was achieved.

Column chromatography was performed on Merck silica gel 60 (230-400 mesh) unless otherwise stated. Radial chromatography was performed on a Harrison Research 7924T Chromatotron using rotors coated with 1, 2 or 4 mm layers of Merck silica gel 60 PF<sub>254</sub>. All coated rotors were dried at 60°C for at least 4 hours before use, and eluted with MeOH after use. Normal phase thin layer chromatography (TLC) was performed on precoated glass plates (Merck silica gel F<sub>254</sub>). Reverse phase TLC was performed on Merck HPTLC RP-8 F<sub>254</sub> plates. Chromatograms were visualised under short wavelength UV light, or by spraying with 13% (w/v) vanillin in sulphuric acid, followed by heating at ca 200°C. Eluent mixtures are reported as the % v/v of the minor constituent.

Melting points were determined on a Reichert hot-stage microscope and both melting and boiling points are uncorrected.

<sup>1</sup>H NMR and <sup>13</sup>C spectra were recorded on the following instruments: Varian CXP20 (<sup>1</sup>H NMR 80 MHz), Jeol JNM FX-200 (<sup>1</sup>H NMR 200 MHz, <sup>13</sup>C NMR 50.1 MHz), Varian XL-200 (<sup>1</sup>H NMR 200 MHz, <sup>13</sup>C NMR 50.3 MHz), Gemini-300 (<sup>1</sup>H NMR 300 MHz, <sup>13</sup>C NMR 75.5 MHz), Varian VXR-300 (<sup>1</sup>H NMR 300 MHz, <sup>13</sup>C NMR 75.4 MHz) and Varian VXR-500 (<sup>1</sup>H NMR 500 MHz). <sup>1</sup>H NMR spectra have been recorded in CDCl<sub>3</sub> using the residual CHCl<sub>3</sub> peak  $\delta$  7.26 ppm as a reference because of the extensive use of silyl ethers. For <sup>13</sup>C NMR spectra recorded in CDCl<sub>3</sub>, the centre peak of the solvent triplet was set at 77.0 ppm. Chemical shifts ( $\delta$ ) are quoted in ppm on the <sup>1</sup>H NMR and <sup>13</sup>C scales. The following abbreviations are used to denote multiplicities and assignments: s (singlet), d (doublet), 'd' (pseudo doublet), dd (doublet of doublets), ddd (doublet of doublet of doublets), t (triplet), dt (doublet of triplets) q (quartet or quaternary carbon), m (multiplet), br (broad) and \* (interchangeable assignments). Acid-free CDCl<sub>3</sub>, where required, was prepared by passing CDCl<sub>3</sub> through a 2 x 0.5 cm column of basic alumina (Merck 90, 70-230 mesh) immediately before use. Distortionless enhancement by polarisation transfer (DEPT) and the attached proton test (APT) were used in the assignment of <sup>13</sup>C spectra.

Two dimensional NMR experiments were performed on either a Varian VXR300 or a VXR500 spectrometer. The pulse sequences used were: homonuclear



$^1\text{H}/^1\text{H}$  correlation spectroscopy (COSY), heteronuclear  $^1\text{H}/^{13}\text{C}$  correlation spectroscopy (HETCOR) and phase sensitive nuclear Overhauser and exchange spectroscopy (NOESY).<sup>181</sup>

Infra-red spectra were recorded on a Perkin Elmer 683 or a Perkin Elmer 1800 (FT) spectrophotometer using solutions in spectroscopic-grade solvents. Peak intensities and shapes are abbreviated as follows: s (strong), m (medium), w (weak), br (broad), sh (shoulder).

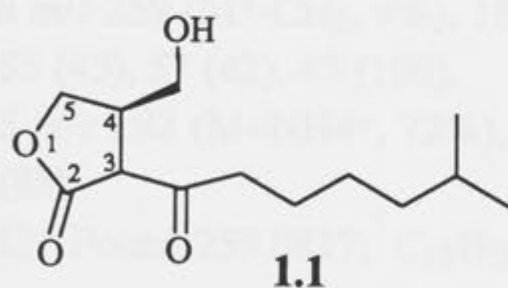
Ultraviolet spectra were recorded on a Varian DMS 90 or a Hewlett-Packard 8450A spectrophotometer using solutions in spectroscopic-grade MeOH unless otherwise stated.

Mass spectra were recorded on AEI 902 and V.G.-Micromass 7070 double focussing mass spectrometers. High resolution mass measurements were obtained from the AEI 902 instrument. The molecular ion ( $M^+$ ) and fragment ions are reported as their mass-to-charge ratios ( $m/z$ ) followed by their relative intensities, as compared with the base (100%) peak.

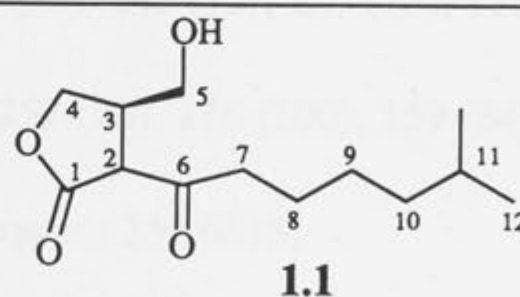
Microanalyses were carried out by the Australian National University Microanalytical Service.

## NOMENCLATURE AND NUMBERING

A-factor and related butanolides are named in Chemical Abstracts as 3,4-disubstituted dihydro-2(3*H*)-furanones where the ring oxygen is the '1' position (see below). However, for consistency with the preceding text and with the nomenclature used in the general chemical literature relating to the butanolide autoregulators, NMR assignments are quoted using the continuous numbering shown for A-factor (**1.1**) below, and on various structures in this section. All butanolides and related compounds in this section are named using a system based on the parent butanolide ring in which the ring carbonyl carbon is the '1' position. All other compounds are named and numbered in accordance with IUPAC nomenclature.



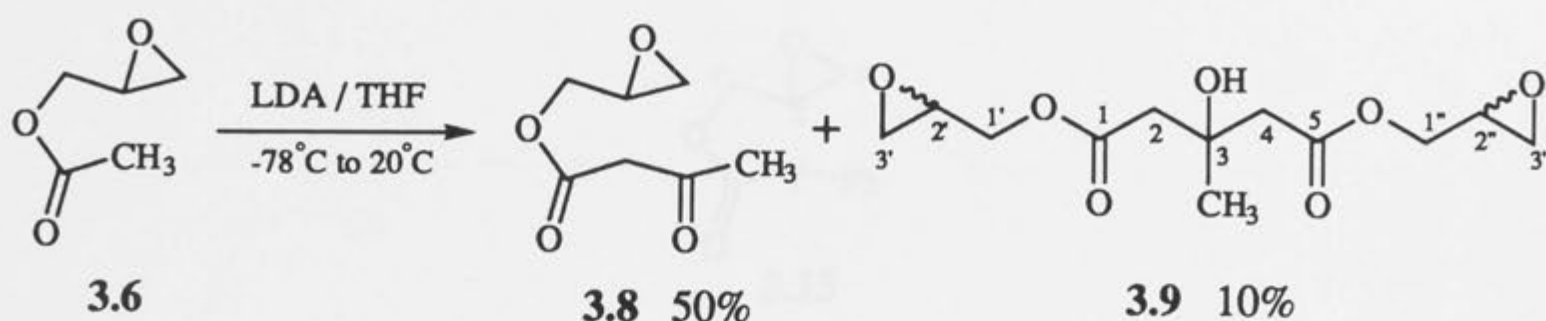
Chemical Abstracts Service nomenclature  
(4*R*)-dihydro-4-(hydroxymethyl)-  
3-(6-methyl-1-oxoheptyl)-2(3*H*)-furanone



Nomenclature used throughout this thesis  
(3*R*)-3-(hydroxymethyl)-2-(6-methyl-  
1-oxoheptyl)-butanolide



## Cyclisation of Oxiranylmethyl acetate (3.6)



To a cooled (0°C) ether solution (5 mL) of diisopropylamine (108 mg, 1.06 mmol) under an argon atmosphere, was added a solution of *n*-butyllithium in hexane (0.64 mL, 1.6 M). After stirring for 20 min, the mixture was cooled further to -78°C and glycidyl acetate<sup>226</sup> (100 mg, 0.86 mmol) in anhydrous ether (1 mL) was slowly introduced. The mixture was allowed to slowly warm to 20°C over 4 hours, before it was quenched by the addition of aqueous phosphate buffer solution (pH 3.5). Extraction with CH<sub>2</sub>Cl<sub>2</sub> (4 x 2 mL), drying and evaporation of the solvent gave a pale yellow oil. Radial chromatography (1 mm, SiO<sub>2</sub>; 0-5% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) separated two major products followed by a complex mixture of polar compounds.

The first eluted compound was oxiranylmethyl 3-oxobutanoate (3.8) (34 mg, 50%), isolated as a colourless oil. <sup>1</sup>H and <sup>13</sup>C NMR data for this compound identical to that reported by Witzeman and Nottingham.<sup>227</sup>

The second eluted product was a diastereomeric mixture of the di(oxiranylmethyl) 3-hydroxy-3-methylpentanedioate (3.9) (7.8 mg, 10%) as a colourless oil:

<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) δ 1.39 (s, 3H, CH<sub>3</sub>), 2.67 (dd, *J*<sub>3'a,3'b</sub> and *J*<sub>3''a,3''b</sub> = 4.8 Hz, *J*<sub>3'b,2'</sub> and *J*<sub>3''b,2''</sub> = 2.6 Hz, 2H, H3'b, H3''b), 2.72 (d, *J*<sub>2a,2b</sub> and *J*<sub>4a,4b</sub> = 5.0 Hz, 4H, H2, H4), 2.86 (dd, *J*<sub>3'a,3'b</sub> and *J*<sub>3''a,3''b</sub> = 4.8 Hz, *J*<sub>3'a,2'</sub> and *J*<sub>3''a,2''</sub> = 4.2 Hz, 2H, H3'a, H3''a), 3.24 (m, 2H, H2', H2''), 3.93 (s, 1H, OH), 3.97 (dd, *J*<sub>1'a,1'b</sub> and *J*<sub>1''a,1''b</sub> = 12.2 Hz, *J*<sub>1'b,2'</sub> and *J*<sub>1''b,2''</sub> = 6.1 Hz, 2H, H1'b, H1''b), 4.47 (dd, *J*<sub>1'a,1'b</sub> and *J*<sub>1''a,1''b</sub> = 12.2 Hz, *J*<sub>1'a,2'</sub> and *J*<sub>1''a,2''</sub> = 3.0 Hz, 2H, H1'a, H1''a).

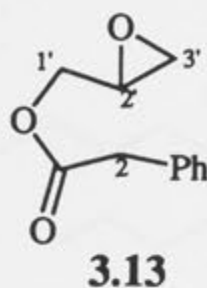
EIMS *m/z* 259 (M<sup>+</sup>-CH<sub>3</sub>, 9%), 185 (5), 183 (6), 159 (38), 143 (12), 127 (12), 116 (20), 85 (45), 57 (42), 43 (100).

CIMS *m/z* 292 (M+NH<sub>4</sub><sup>+</sup>, 72%), 275 (M+H<sup>+</sup>, 61), 257 (20), 176 (100), 159 (84), 117 (88).

HRMS: Found 259.0817; C<sub>15</sub>H<sub>29</sub>O<sub>4</sub>Si (M<sup>+</sup>-CH<sub>3</sub>) requires 259.0818.

The last band eluted contained a mixture of several unidentified compounds which appeared by <sup>1</sup>H NMR spectroscopy to contain intact epoxide moieties, as indicated by complex resonances at near δ 4.5, 4.0, 3.2, 2.9 and 2.7.

## Oxiranylmethyl benzeneacetate (3.13)



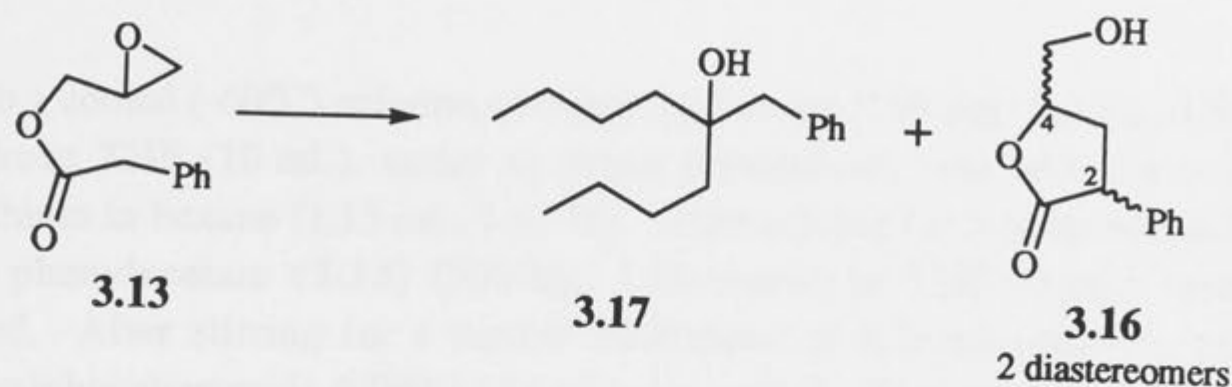
The glycidyl ester (3.13) was prepared according to the method of Fuganti and co-workers.<sup>102</sup> The crude product was purified by flash chromatography (20% hexane in  $\text{CH}_2\text{Cl}_2$  in 69% yield as pale yellow oil, bp 92-95°C / 0.2 mm Hg.

$^1\text{H}$  NMR (80 MHz,  $\text{CDCl}_3$ )  $\delta$  2.56 (dd,  $J_{3'a,3'b} = 4.9$  Hz,  $J_{3'b,2'} = 2.5$  Hz, 1H, H3'b), 2.77 (dd,  $J_{3'a,3'b} = 4.9$  Hz,  $J_{3'a,2'} = 4.2$  Hz, 1H, H3'a), 3.1-3.3 (m, 1H, H2'), 3.65 (s, 2H, H2), 3.92 (dd,  $J_{1'a,1'b} = 12.3$  Hz,  $J_{1'b,2'} = 6.0$  Hz, 1H, H1'b), 4.41 (dd,  $J_{1'a,1'b} = 12.3$  Hz,  $J_{1'a,2'} = 3.1$  Hz, 1H, H1'a), 7.28 (s, 5H, ArH).

EIMS  $m/z$  192 (M, 5%), 136 (4.5), 118 (48), 91 (100), 86 (47), 84 (70), 65 (12).



## Cyclisation of Oxiranylmethyl benzeneacetate (3.13)

METHOD A: USING LDA WITH *n*-BUTYLLITHIUM

To a cooled ( $-30^{\circ}\text{C}$ ) THF solution (20 mL) of diisopropylamine (87 mg, 121  $\mu\text{L}$ , 0.86 mmol) under an argon atmosphere, was added a solution of *n*-butyllithium in hexane (0.56 mL, 1.55 M). After stirring for 5 min, the glycidyl ester (3.13) (150 mg, 0.78 mmol) in THF (3 mL) was introduced. The mixture was allowed to warm to  $20^{\circ}\text{C}$  and was stirred for 1 hour. A sample analysed by TLC (2% MeOH in  $\text{CH}_2\text{Cl}_2$ ) indicated only starting material. After re-cooling to  $-30^{\circ}\text{C}$  an additional equivalent of *n*-butyllithium in hexane (0.56 mL, 1.55 M) was added. This mixture was stirred at  $10^{\circ}\text{C}$  for 2 hours before being quenched by the addition of pH 3.5 phosphate buffer (3 mL) and water (5 mL). Extraction with  $\text{CH}_2\text{Cl}_2$  (4 x 5 mL), drying and evaporation of the solvent gave a pale yellow oil. Radial chromatography (2 mm,  $\text{SiO}_2$ ; 0-5% MeOH in  $\text{CH}_2\text{Cl}_2$ ) separated two major products followed by a complex mixture of polar compounds.

The first eluted compound was  $\alpha,\alpha$ -dibutylbenzeneethanol (3.17) (38 mg, 21%) isolated as a colourless oil:

$^1\text{H}$  NMR (80 MHz,  $\text{CDCl}_3$ )  $\delta$  0.91 (m, 6H, 2 x  $\text{CH}_3$ ), 1.37 (m, 12H, 6 x  $\text{CH}_2$ ), 2.74 (s, 2H,  $\text{CH}_2\text{Ar}$ ), 7.25 (m, 5H, ArH).

EIMS  $m/z$  177 ( $\text{M}^+ - \text{C}_4\text{H}_9$ , 24%), 143 ( $\text{M}^+ - \text{CH}_2\text{Ar}$ , 100), 92 (37), 91 ( $\text{ArCH}_2^+$ , 60), 85 (26), 83 (26), 69 (85), 57 (63), 55 (46).

The second eluted product was a diastereomeric mixture of the 4-hydroxymethyl-2-phenylbutanolides (3.16) (60 mg, 40%) as colourless needles from hexane /  $\text{CH}_2\text{Cl}_2$ , mp  $82-91^{\circ}\text{C}$ :

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  2.33-2.50 (m, 2H, 2 x H3b), 2.60-2.73 (m, 2H, 2 x H3a), 3.69 (t,  $J = 5$  Hz, 1H, H2), 3.73 (t,  $J = 5$  Hz, 1H, H2), 3.90-4.07 (m, 4H, 2 x H5), 4.60-4.67 (m, 1H, H4), 4.70-4.76 (m, 1H, H4), 7.25-7.43 (m, 10H, ArH).

$^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ )  $\delta$  32.4 (C3), 32.7 (C3), 46.1 (C2), 46.9 (C2), 63.2 (C5), 64.1 (C5), 78.8 (2 x C4), 127.0, 127.2, 127.3, 127.8, 128.3, 128.5, 136.9, 137.8 (12 x ArC), 177.1, 178.1 (2 x C=O).

IR ( $\text{CCl}_4$ )  $\nu_{\text{max}}$  3600 m (OH), 1771 s (lactone C=O), 1500 m, 1453 m, 1358 m, 1170 br s  $\text{cm}^{-1}$ .

EIMS  $m/z$  192 ( $\text{M}^+$ , 34%), 161 ( $\text{M}^+ - \text{CH}_2\text{OH}$ , 9), 148 ( $\text{M}^+ - \text{CO}_2$ , 12), 133 (29), 118 (23), 117 (100), 115 (20), 105 (74), 104 (47), 91 (21), 77 (22).



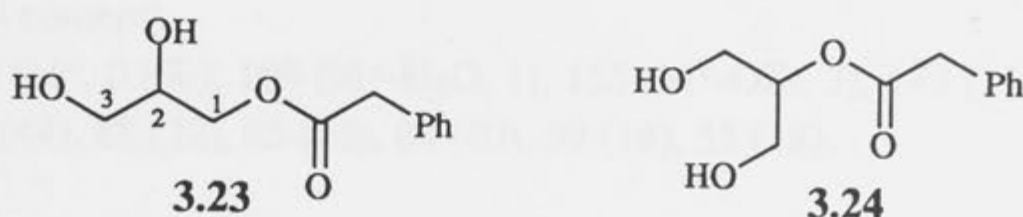
Analysis: Found C 68.2, H 6.4;  $C_{11}H_{12}O_3$  requires C 68.5, H 6.3.

*METHOD B: USING LDA IN HMPA-THF (1:10)*

To a cooled ( $-60^{\circ}\text{C}$ ) solution of diisopropylamine (190 mg, 264  $\mu\text{L}$ , 1.87 mmol) in anhydrous THF (10 mL), under an argon atmosphere, was added a solution of *n*-butyllithium in hexane (1.15 mL, 1.61 M). After stirring for 5 minutes a solution of glycidyl phenylacetate (**3.13**) (300 mg, 1.56 mmol) in THF (3 mL) was slowly introduced. After stirring for a further 30 minutes at this temperature, anhydrous hexamethylphosphoramide (HMPA) (1 mL) was added. The mixture was allowed to warm to  $0^{\circ}\text{C}$  and stirred for 4 hours, after which time no starting material was detected by TLC (5% MeOH in  $\text{CH}_2\text{Cl}_2$ ). The reaction was quenched by the addition of phosphate buffer (pH 3.5, 3 mL) and water (3 mL). Approximately half of the THF was removed by rotary evaporation before extraction with EtOAc (3 x 7 mL). The extracts were washed with saturated aqueous copper sulphate solution (3 x 3 mL) to remove HMPA, followed by water (2 x 5 mL). Drying and evaporation of the solvent yielded an oil from which two products were isolated using radial chromatography (2 mm  $\text{SiO}_2$ ; 0-8% MeOH in  $\text{CH}_2\text{Cl}_2$ ), as well as a complex mixture of highly polar compounds.

The first eluted product was the previously identified diastereomeric mixture of 4-hydroxymethyl-2-phenylbutanolides (**3.16**) (75 mg, 25%) (see Method A above for spectral data).

The second eluted product was an inseparable mixture (5:1) of 2,3-dihydroxypropyl phenylacetate (**3.23**) and 1,3-dihydroxypropyl phenylacetate (**3.24**), (98 mg, 30% combined) as a colourless oil.



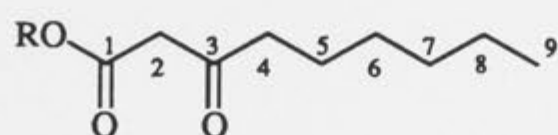
$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) for diol **3.23**:  $\delta$  3.48 (dd,  $J_{3a,3b} = 11.7$  Hz,  $J_{3b,2} = 6.0$  Hz, 1H, H3b), 3.60 (dd,  $J_{3a,3b} = 11.7$  Hz,  $J_{3a,2} = 3.7$  Hz, 1H, H3a), 3.65 (s, 2H,  $\text{CH}_2\text{Ar}$ ), 3.80-3.92 (m, 1H, H2), 4.13 (d,  $J = 5.5$  Hz, 2H, H1), 7.40-7.20 (m, 5H, ArH); for diol **3.24**:  $\delta$  3.74 (d,  $J = 4.8$  Hz, 4H, H1,3), 4.85 (m, 1H, H2) (other peaks obscured by **3.23**).

$^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) (for diol **3.23**)  $\delta$  41.0 ( $\text{CH}_2\text{Ar}$ ), 63.1 (C3), 65.5 (C2), 70.0 (C1), 127.1, 128.4, 129.1, 133.4 (6 x ArC), 172.5 (C=O); for diol **3.24**:  $\delta$  61.4 (C1, C3), 75.5 (C2), other peaks coincident with those of **3.23**.

EIMS  $m/z$  210 ( $\text{M}^+$ , 1%), 179 ( $\text{M}^+ - \text{CH}_2\text{OH}$ , 5), 135 (8), 118 (9), 91 (67), 86 (60), 84 (100), 49 (28).



## Methyl 3-oxononanoate (3.33a)



3.33a R = Me

3.33b R = Et

3.33c R = H

3.33d R = *t*Bu

The methyl  $\beta$ -ketoester (3.33a) was prepared from ethyl acetoacetate and 1-bromopentane by the method of Stallberg-Stenhagen.<sup>119</sup> Fraction distillation yielded the product in 60% yield, bp 77-82°C / 1 mm Hg. [Lit.<sup>228</sup> bp 137°C / 28 mm Hg].

<sup>1</sup>H NMR (80 MHz, CDCl<sub>3</sub>)  $\delta$  0.88 (m, 3H, H<sub>9</sub>) 1.1-1.9 (m, 8H, H<sub>5,6,7,8</sub>), 2.25 (t,  $J$  = 5 Hz, enol H<sub>4</sub>), 2.53 (t,  $J$  = 7 Hz, 2H, H<sub>4</sub>), 3.44 (s, 2H, H<sub>2</sub>), 3.73 (s, 3H, OMe), 5.00 (s, enol CH), 11.3 (s, enol OH), [10% enol content].

EIMS  $m/z$  186 (M<sup>+</sup>, 3.4%), 168 (M<sup>+</sup>-H<sub>2</sub>O, 2), 155 (M<sup>+</sup>-OMe, 3), 144 (4), 143 (4), 129 (26), 116 (100), 113 (62), 101 (45), 97 (22), 85 (37), 84 (95), 74 (58), 69 (52), 59 (45), 57 (43), 56 (27), 55 (46).

## Ethyl 3-oxononanoate (3.33b)

The ethyl  $\beta$ -ketoester (3.33b) was prepared by alkylation of the dianion of ethyl acetoacetate with 1-bromopentane using the procedure of Huckin and Weiler.<sup>118</sup> Fractional distillation of the crude product afforded the title compound in 65% yield, (bp 68-69°C / 0.25 mm Hg [Lit.<sup>229</sup> bp 87°C / 0.8 mm Hg]).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.88 (m, 3H, H<sub>9</sub>) 1.28 (m, 9H, H<sub>6,7,8</sub> + ethyl CH<sub>3</sub>), 1.60 (m, 2H, H<sub>5</sub>), 2.19 (t,  $J$  = 7 Hz, enol H<sub>4</sub>), 2.54 (t,  $J$  = 7 Hz, 2H, H<sub>4</sub>), 3.43 (s, 2H, H<sub>2</sub>), 4.20 (q,  $J$  = 7 Hz, 2H ethyl CH<sub>2</sub>), 4.97 (s, enol CH), 12.11 (s, enol OH), [10% enol content].

EIMS  $m/z$  200 (M<sup>+</sup>, 0.8%), 168 (M<sup>+</sup>-H<sub>2</sub>O, 1), 155 (M<sup>+</sup>-OEt, 3), 143 (11), 130 (51), 115 (11), 113 (44), 88 (22), 85 (16), 84 (40), 59 (16), 55 (18).

*t*-Butyl 3-oxononanoate (3.33d)

The title ester (3.33d) was prepared by alkylation of the dianion of *t*-butylacetoacetate with 1-bromopentane using the method of Huckin and Weiler.<sup>118</sup> Fractional distillation of the crude product afforded *t*-butyl 3-oxononanoate in 70% yield; bp 80-82°C / 0.1 mm Hg [Lit.<sup>162</sup> bp 88°C / 0.15 mm Hg].

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.85 (m, 3H, H<sub>9</sub>), 1.20-1.35 (m, 6H, H<sub>6,7,8</sub>), 1.44 (s, 9H, *t*-butyl), 1.56 (m, 2H, H<sub>5</sub>), 2.49 (t,  $J$  = 7 Hz, 2H, H<sub>4</sub>), 3.31 (s, 2H, H<sub>2</sub>), 4.85 (s, enol CH), 12.1 (s, enol OH), [6% enol content].

<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  13.9 (C<sub>9</sub>), 22.4 (C<sub>8</sub>), 23.4 (C<sub>5</sub>), 27.9 (*t*Bu), 28.7 (C<sub>6</sub>), 31.5 (C<sub>7</sub>), 42.9 (C<sub>4</sub>), 50.6 (C<sub>2</sub>), 81.8 (q, *t*Bu), 166.5 (C<sub>3</sub>), 203.4 (C<sub>1</sub>).

EIMS  $m/z$  172 (M<sup>+</sup>-C<sub>4</sub>H<sub>8</sub>, 2%), 155 (3), 113 (17), 102 (18), 85 (8), 84 (16), 59 (12), 58 (12), 57 (100), 55 (10).

Analysis: Found C 68.3, H 10.9; C<sub>13</sub>H<sub>24</sub>O<sub>3</sub> requires C 68.4, H 10.6.



**3-Oxononanoic acid (3.33c)***METHOD A*

The title acid was prepared *via* acid hydrolysis of the methyl (3.33a) or ethyl  $\beta$ -ketoesters (3.33b) by a procedure based on that of Mitz and coworkers.<sup>120</sup> To the 3-oxononanoate ester (3.33a or 3.33b, 5.0 g) in glacial acetic acid (80 mL) was added concentrated hydrochloric acid (40 mL, 36%). The solution was stirred at 20°C for 20 hours before it was concentrated on a rotary evaporator (temp < 30°C). After about 80 mL of solvent had been removed the product crystallised. Further crystallisation was promoted by cooling in ice before the  $\beta$ -ketoacid was collected by filtration and washed well with water (3 x 30 mL). Concentration of the filtrate yielded additional crops of product which were combined and dried *in vacuo* over P<sub>2</sub>O<sub>5</sub>, to give 3-oxononanoic acid (3.33c) as colourless plates (2.8 g, 61% from 3.33a; 2.58 g, 60% from 3.33b), mp 70-71°C (Lit.<sup>230</sup> mp 70.5°C).

If the product is to be stored for an extended period it is recommended that it be recrystallised from benzene-hexane (mp 70.5-71.0°C) and stored at low temperature to minimise the risk of decarboxylation.

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.87 (m, 3H, H<sub>9</sub>), 1.20-1.37 (m, 6H, H<sub>6,7,8</sub>), 1.52-1.67 (m, 2H, H<sub>5</sub>), 2.21 (t, *J* = 7 Hz, enol H<sub>4</sub>), 2.56 (t, *J* = 7 Hz, 2H, H<sub>4</sub>), 3.50 (s, 2H, H<sub>2</sub>), 5.02 (s, enol CH), 10.2 (br s, 1H, OH), 11.5 (s, enol OH), [18% enol content].

<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  14.0 (C<sub>9</sub>), 22.4 (C<sub>5</sub>)\*, 23.3 (C<sub>8</sub>)\*, 28.6 (C<sub>6</sub>), 31.5 (C<sub>7</sub>), 35.3 (C<sub>4</sub> enol), 43.2 (C<sub>4</sub>), 48.1 (C<sub>2</sub>), 88.2 (C<sub>2</sub>), 172.2 (C<sub>1</sub>), 176.9 (C<sub>1</sub> enol), 182.2 (C<sub>3</sub> enol), 203.9 (C<sub>3</sub>).

EIMS *m/z* 172 (M<sup>+</sup>, 0.4%), 128 (M<sup>+</sup>-CO<sub>2</sub>, 7), 113 (5), 102 (11), 85 (8), 84 (14), 71 (29), 58 (100).

*METHOD B*

To *t*-butyl 3-oxononanoate (3.33d) (14.8 g, 65 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (60 mL) was added trifluoroacetic acid (40 mL). The mixture was stirred for 2 hours at room temperature before the solvents were removed using a rotary evaporator. Solvent traces were then removed under high vacuum (0.1 mm Hg) to leave a crystalline mass. Recrystallisation from pentane gave 3-oxononanoic acid (8.85 g, 80%) in two crops, mp 69.5-70°C (spectroscopic data above).



## Oxiranylmethyl 3-oxononanoate (3.32a)



To a cooled (0°C) solution of 3-oxononanoic acid (3.33c) (1.49 g, 8.7 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (45 mL) was added glycidol (3.4) (0.71 g, 9.6 mmol) and *N,N*-dicyclohexylcarbodiimide (DCC) (1.98 g, 9.6 mmol) followed 5 minutes later by DMAP (50 mg) and DMAP hydrochloride salt (50 mg). After stirring overnight at room temperature the resulting white suspension was concentrated by the evaporation of *ca* 30 mL of solvent with a stream of dry argon. After cooling to 0°C the precipitated *N,N*-dicyclohexylurea was removed by filtration and the filtrate was washed with pH 5.0 phosphate buffer (2 x 15 mL) and water (3 x 15 mL). Drying and evaporation of the solvent gave a pale yellow oil which was purified by Kugelrohr distillation (oven temp. 80-90°C / 0.1 mm Hg) yielding the  $\beta$ -ketoester (3.32a) as a colourless oil (1.40 g, 70%).

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.87 (t,  $J = 6.8$  Hz, 3H, H9), 1.20-1.38 (m, 6H, H6,7,8), 1.59 (m, 2H, H5), 2.20 (t,  $J = 7$ , Hz, enol H4), 2.53 (t,  $J = 7.4$  Hz, H4), 2.66 (dd,  $J_{3'a,3'b} = 4.9$  Hz,  $J_{3'b,2'} = 2.6$  Hz, 1H, H3'b), 2.85 (dd,  $J_{3'a,3'b} = 4.9$  Hz,  $J_{3'b,2'} = 4.0$  Hz, 1H, H3'a), 3.22 (m, 1H, H2'), 3.50 (s, 2H, H2), 3.99 (dd,  $J_{1'a,1'b} = 12.3$  Hz,  $J_{1'b,2} = 6.2$  Hz, 1H, H1'b), 4.47 (dd,  $J_{1'a,1'b} = 12.3$  Hz,  $J_{1'a,2'} = 3.0$  Hz, 1H, H1'a), 5.03 (s, enol H2), 11.87 (s, enol OH), (enol content *ca* 10%)

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  13.9 (C9), 22.4 (C8), 23.3 (C5), 28.6 (C6), 31.4 (C7), 43.1 (C4), 44.6 (C3'), 48.8 (C2), 49.0 (C2'), 65.5 (C1'), 166.8 (ester C=O), 202.4 (ketone C=O).

IR ( $\text{CCl}_4$ ),  $\nu_{\text{max}}$  2930 s, 1755 s (ester C=O), 1723 s (ketone C=O), 1660 s, 1630 s, 1222 s, 1150 m  $\text{cm}^{-1}$ .

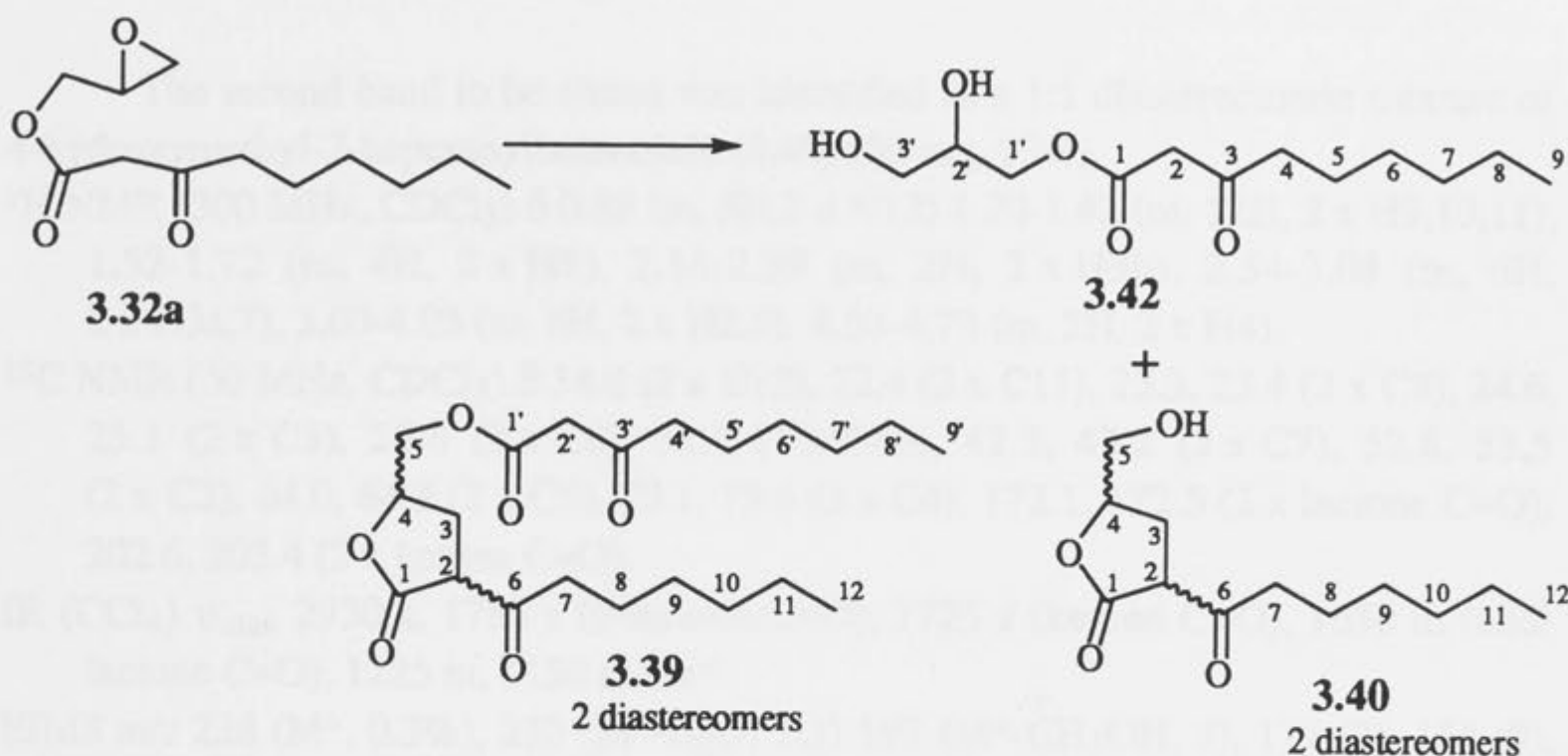
EIMS  $m/z$  171 ( $\text{M}^+ - \text{C}_4\text{H}_9$ , 2%), 158 (4), 116 (McLafferty rearrangement, 41), 113 (28), 84 (51), 69 (19), 57 (29), 43 (100).

CIMS  $m/z$  246 ( $\text{M} + \text{NH}_4^+$ , 25%), 229 ( $\text{M} + \text{H}^+$ , 100).

Analysis: Found C 63.2, H 8.7;  $\text{C}_{12}\text{H}_{20}\text{O}_4$  requires C 63.1, H 8.8.



## Cyclisation of Oxiranylmethyl 3-oxononanoate (3.32a)



Mineral oil was removed from sodium hydride (50% oil dispersion, 126 mg, 2.6 mmol) by washing and decanting twice with dry pentane (2 x 3 mL) under an atmosphere of anhydrous argon. The hydride was suspended in dry THF (6 mL) and cooled to 0°C before a solution of the  $\beta$ -ketoester (3.32a) (500 mg, 2.19 mmol) in THF (10 mL) was introduced dropwise. Stirring for 30 min at room temperature resulted in no reaction as determined by reverse phase TLC (RP8; 20% H<sub>2</sub>O in MeOH). After heating at reflux for 3 hours the reaction mixture was quenched by the addition of a phosphate buffer solution (pH 5.0, 5 mL) and then extracted with ether (3 x 10 mL). The organic extracts were washed with water (2 x 5 mL), dried and evaporated to give a yellow oil. Radial chromatography (4 mm SiO<sub>2</sub>; 1-5% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) separated three products with the remainder being complex polar material.

The first eluted band was identified as a 1:1 diastereomeric mixture of the O-(3'-oxononanoyl) butyrolactones (3.39) (175 mg, 42%).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.88 (m, 12H, 2 x H<sub>12,9'</sub>), 1.19-1.41 (m, 24H, 2 x H<sub>9,10,11,6',7',8'</sub>), 1.5-1.7 (m, 4H, 2 x H<sub>8</sub>), 2.07-2.43 (m, 2H, 2 x H<sub>3b</sub>), 2.52 (t,  $J = 8$  Hz, 4H, 2 x H<sub>4'</sub>), 2.57-3.08 (m, 6H, 2 x H<sub>3a,7</sub>), 3.49 (s, 2H, H<sub>2'</sub>), 3.51 (s, 2H, H<sub>2'</sub>), 3.78 (dd,  $J = 8.8$  Hz,  $J = 10.0$  Hz, 1H, H<sub>2</sub>), 3.84 (dd,  $J = 5.5$  Hz,  $J = 9.6$  Hz, 1H, H<sub>2</sub>), 4.20-4.43 (m, 4H, 2 x H<sub>5</sub>), 4.67-4.84 (m, 2H, 2 x H<sub>4</sub>).

<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  13.9 (2 x C<sub>12</sub>, 2 x C<sub>9'</sub>), 22.3 (2 x C<sub>11</sub>, 2 x C<sub>8'</sub>), 23.1, 23.2, 23.3 (2 x C<sub>8</sub>, 2 x C<sub>5'</sub>), 24.8 (C<sub>3</sub>), 25.4 (C<sub>3</sub>), 28.5 (2 x C<sub>9</sub>, 2 x C<sub>6'</sub>), 31.4 (2 x C<sub>10</sub>, 2 x C<sub>7'</sub>), 42.1, 42.3 (2 x C<sub>7</sub>)\*, 43.0, 43.2 (2 x C<sub>4'</sub>)\*, 48.6, 48.8 (2 x C<sub>2'</sub>), 52.3, 52.7 (2 x C<sub>2</sub>), 65.4 (2 x C<sub>5</sub>), 75.7, 76.4 (2 x C<sub>4</sub>), 166.7, 166.9 (2 x ester C=O), 171.7, 171.8 (2 x lactone C=O), 202.0, 202.3, 202.6 (4 x ketone C=O).

IR (CCl<sub>4</sub>)  $\nu_{\max}$  2935 s, 1782 s ( $\gamma$ -lactone C=O), 1758 s (ester C=O), 1725 s (ketone C=O), 1410 m, 1150 m cm<sup>-1</sup>.



EIMS  $m/z$  228 ( $M^+ - C_9H_{15}O_2$ , 0.3%), 210 (1), 197 (1), 171(2), 158 (4), 116 (40), 113 (23), 84 (41), 69 (12), 57 (31), 55 (21), 42 (100).

CIMS  $m/z$  400 ( $M + NH_4^+$ , 100%).

The second band to be eluted was identified as a 1:1 diastereomeric mixture of 4-hydroxymethyl-2-heptanoylbutanolide (**3.40**) (50 mg, 10%).

$^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  0.88 (m, 6H, 2 x H12) 1.20-1.42 (m, 12H, 2 x H9,10,11), 1.52-1.72 (m, 4H, 2 x H8), 2.16-2.39 (m, 2H, 2 x H3b), 2.54-3.08 (m, 6H, 2 x H3a,7), 3.60-4.03 (m, 6H, 2 x H2,5), 4.58-4.73 (m, 2H, 2 x H4).

$^{13}C$  NMR (50 MHz,  $CDCl_3$ )  $\delta$  14.0 (2 x C12), 22.4 (2 x C11), 23.3, 23.4 (2 x C8), 24.6, 25.1 (2 x C3), 28.6 (2 x C9), 31.5 (2 x C10), 42.3, 43.2 (2 x C7), 52.8, 53.5 (2 x C2), 64.0, 64.3 (2 x C5), 79.1, 79.6 (2 x C4), 172.1, 172.5 (2 x lactone C=O), 202.6, 203.4 (2 x ketone C=O).

IR ( $CCl_4$ )  $\nu_{max}$  2930 s, 1780 s ( $\gamma$ -lactone C=O), 1725 s (ketone C=O), 1650 m (enol lactone C=O), 1225 m, 1150  $cm^{-1}$ .

EIMS  $m/z$  228 ( $M^+$ , 0.3%), 210 ( $M^+ - H_2O$ , 1.5) 197 ( $M^+ - CH_2OH$ , 3), 171 (2), 158 (9), 116 (33), 113 (98), 85 (30), 70 (11), 57 (20), 55 (42), 43 (100),

CIMS  $m/z$  246 ( $M + NH_4^+$ , 100%), 229 ( $M + H^+$ , 60).

HRMS: Found 228.1362;  $C_{12}H_{20}O_4$  ( $M^+$ ) requires 228.1362.

The third and final band eluted was 2',3'-dihydroxypropyl 3-oxononanoate (**3.42**), (81 mg, 15%):

$^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  0.88 (m, 3H, H9) 1.18-1.38 (m, 6H, H6,7,8), 1.50-1.66 (m, 2H, H5), 2.52 (t,  $J = 7.2$  Hz, 2H, H4), 3.51 (s, 2H, H2), 3.61 (dd,  $J_{3'a,3'b} = 11.5$  Hz,  $J_{3'b,2'} = 5.7$  Hz, 1H, H3'b), 3.60 (dd,  $J_{3'a,3'b} = 11.5$  Hz,  $J_{3'a,2'} = 4.1$  Hz, 1H, H3'a), 3.95 (m, 1H, H2'), 4.25 (d,  $J = 5.4$  Hz, 2H, H1').

EIMS  $m/z$  215 ( $M^+ - CH_2OH$ , 3%), 186 (2), 173 (4), 158 (16), 155 (6), 116 (22), 113 (55), 85 (17), 84 (42), 69 (14), 61 (14), 57 (21), 55 (14), 43 (100).

HRMS: Found ( $M^+ - CH_2OH$ ) 215.1284;  $C_{11}H_{19}O_4$  requires 215.1284.

**2-Iodoethyl 3-oxononanoate (3.46)**

To a stirred solution of DCC (264 mg, 1.28 mmol) in anhydrous  $\text{CH}_2\text{Cl}_2$  (6 mL) at  $0^\circ\text{C}$  was added 3-oxononanoic acid (**3.33c**) (200 mg, 1.16 mmol) and DMAP hydrochloride (20 mg). After 30 min at  $0^\circ\text{C}$ , 2-iodoethanol (220 mg, 1.28 mmol) was introduced and the mixture was stirred at  $20^\circ\text{C}$  for 20 hours. The suspension was cooled ( $0^\circ\text{C}$ ) and concentrated to *ca* 4 mL in a stream of argon before removal of the precipitated *N,N*-dicyclohexylurea by filtration. The filtrate was washed sequentially with water (10 mL), 5% acetic acid (10 mL) and water (2 x 10 mL) before being dried and evaporated. Radial chromatography of the residue (2 mm  $\text{SiO}_2$ ; 0-1% MeOH in  $\text{CH}_2\text{Cl}_2$ ) allowed the isolation of the iodo  $\beta$ -ketoester (**3.46**) as a lemon-yellow waxy solid (227 mg, 60%).

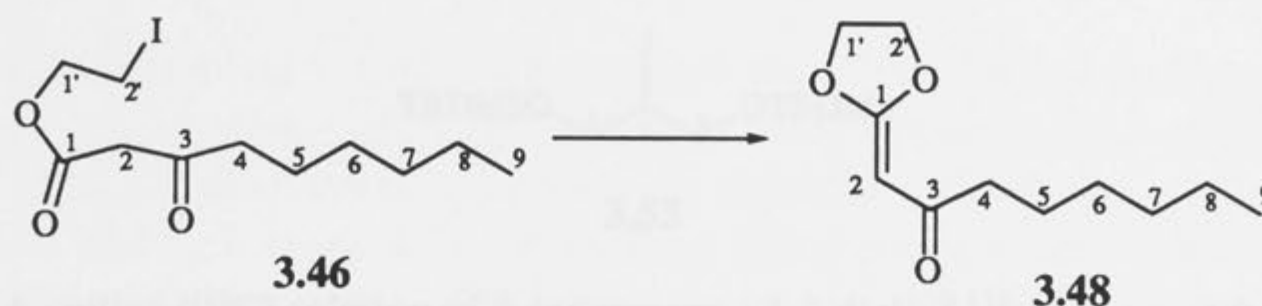
$^1\text{H}$  NMR (80 MHz,  $\text{CDCl}_3$ )  $\delta$  0.88 (m, 3H, H<sub>9</sub>) 1.10-1.90 (m, 8H, H<sub>5,6,7,8</sub>), 2.55 (t,  $J = 7.0$  Hz, H<sub>4</sub>), 3.30 (t,  $J = 6.8$  Hz, 2H, H<sub>2'</sub>), 3.46 (s, 2H, H<sub>2</sub>), 4.39 (t,  $J = 6.8$  Hz, 2H, H<sub>1'</sub>).

EIMS  $m/z$  326 ( $\text{M}^+$ , 0.5%), 298 (0.3), 256 (15), 227 (4), 199 (6), 155 (63), 113 (29), 101 (9), 85 (17), 84 (27), 69 (23), 57 (14), 55 (22), 45 (21), 43 (100).

Analysis: Found C 40.4, H 5.9;  $\text{C}_{11}\text{H}_{19}\text{O}_3\text{I}$  requires C 40.5, H 5.9.



## Cyclisations of 2-Iodoethyl 3-oxononanoate (3.46)



## METHOD A: USING SODIUM HYDRIDE

A solution of the iodo ester (3.46) (94 mg, 0.29 mmol) in anhydrous THF (2 mL) was transferred *via* a cannula to a stirred suspension of sodium hydride (50% oil dispersion, 16.3 mg, 0.34 mmol) in THF (2 mL) at 20°C, under an atmosphere of argon. After 5 min, TLC (SiO<sub>2</sub>; 5% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) indicated the disappearance of starting material so the reaction mixture was quenched by the addition of phosphate buffer (pH 5.0, 1 mL) and water (3 mL). Extraction with CH<sub>2</sub>Cl<sub>2</sub> (3 x 3 mL) and subsequent drying and evaporation of solvent gave a pale yellow oil. Radial chromatography (1 mm SiO<sub>2</sub>; 0-2% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) facilitated the isolation of the ketene acetal (3.48) (43 mg, 75%) as a colourless oil.

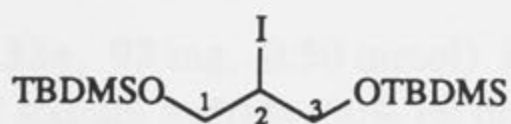
<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) δ 0.88 (m, 3H, H<sub>9</sub>) 1.10-1.45 (m, 6H, H<sub>6,7,8</sub>), 1.45-1.70 (m, 2H, H<sub>5</sub>), 2.40 (t, *J* = 7 Hz, 2H, H<sub>4</sub>), 4.40 (t, *J* = 7 Hz, 2H, H<sub>1'</sub>)\*, 4.62 (t, *J* = 7 Hz, 2H, H<sub>2'</sub>)\*, 4.95 (s, 1H, H<sub>2</sub>).

EIMS *m/z* 198 (M<sup>+</sup>, 2%), 155 (1), 141 (4), 128 (39), 113 (100), 86 (15), 69 (56), 55 (9), 45 (12), 43 (27).

HRMS: Found (M<sup>+</sup>) 198.1256; C<sub>11</sub>H<sub>18</sub>O<sub>3</sub> requires 198.1256.

METHOD B: USING TETRA-*n*-BUTYL AMMONIUM FLUORIDE

The iodoethyl ester (3.46) (79 mg, 0.23 mmol) in anhydrous THF (10 mL) was stirred over crushed molecular sieves (4 Å, 0.8 g) for 1.5 hours at room temperature before a solution of tetra-*n*-butyl ammonium fluoride (1.0 M in THF, 0.5 mmol) was added. Analysis by TLC (1% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) after 5 minutes showed no starting material. The molecular sieves were removed by filtration and phosphate buffer (pH 5.0, 2 mL) was added to the filtrate. Extraction with CH<sub>2</sub>Cl<sub>2</sub> (3 x 5 mL), drying and removal of the solvent left a residue of yellow oil. Radial chromatography (2 mm SiO<sub>2</sub>; 2-4% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) isolated the ketene acetal (3.48) (33 mg, 70%) as a colourless oil. (spectroscopic data above).

**1,3-Bis(*t*-butyldimethylsilyloxy)-2-iodopropane (3.53)****3.53**

A chilled (0°C) solution of 2-iodopropane-1,3-diol<sup>129,130</sup> (404 mg, 2.0 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) and pyridine (0.5 mL) was treated with TBDMS-trifluoromethanesulfonate (1.16 g, 4.4 mmol) and stirred for 10 minutes. The reaction was quenched with saturated aqueous sodium bicarbonate solution (2 mL) and water (5 mL). The aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 5 mL) and the extracts combined with the organic layer. Drying and evaporation of the solvent gave a light yellow oil which was purified by Kugelrohr distillation (oven temp. 90-110°C / 0.3 mm Hg). Yield of the diprotected 2-iodopropanediol (**3.53**) as a colourless liquid is 830 mg (96%).

<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) δ 0.07 (s, 12H, SiCH<sub>3</sub>), 0.90 (s, 18H, *t*Bu), 3.87 (d, *J* = 5.5 Hz, 4H, 2 x H1,3), 4.10 (pentet, *J* = 5.5 Hz, 1H, H2).

<sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ -5.4, -5.3 (SiCH<sub>3</sub>), 18.3 (q, *t*-Bu), 25.8 (*t*Bu), 36.5 (C2), 64.5 (C1,3).

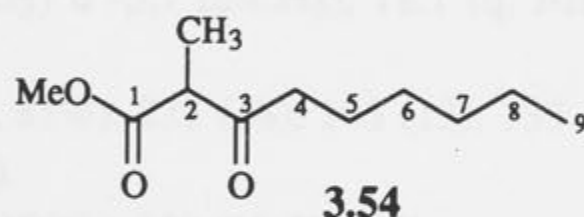
EIMS *m/z* 373 (M<sup>+</sup>-C<sub>4</sub>H<sub>9</sub>, 14%), 259 (5), 215 (4), 189 (18), 147 (66), 133 (17), 131 (56), 115 (14), 101 (10), 89 (34), 75 (19), 73 (100), 59 (21), 57 (11).

HRMS: Found (M<sup>+</sup>-C<sub>4</sub>H<sub>9</sub>) 373.0517; C<sub>11</sub>H<sub>26</sub>O<sub>2</sub>Si<sub>2</sub>I requires 373.0516.



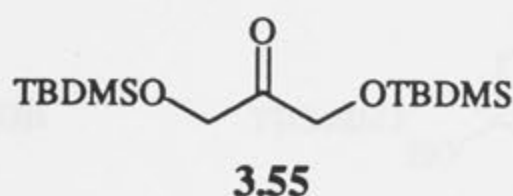
### Attempted Intermolecular Alkylation of Methyl 3-oxononanoate (3.33a) with Iodide 3.53

The  $\beta$ -ketoester (3.33a, 93 mg, 0.50 mmol) in THF (2 mL) was slowly introduced to a suspension of sodium hydride (50% oil dispersion, 28.8 mg, 0.6 mmol) in THF (2 mL) at room temperature under an atmosphere of anhydrous argon. After 10 minutes hydrogen evolution had ceased and a clear solution remained. The iodide (3.53) (215 mg, 0.50 mmol) in THF (1 mL) was then added *via* a cannula and the mixture was heated at reflux for 48 hours. Periodic analysis by TLC (1% MeOH in  $\text{CH}_2\text{Cl}_2$ ) showed no reaction during this time. To confirm formation of the  $\beta$ -ketoester anion the reaction mixture was cooled to 20°C and the anion was 'trapped' by the addition of a large excess of methyl iodide (1.1 g). After 30 min, phosphate buffer (pH 5.0, 1.5 mL) and water (2 mL) were added and the mixture was extracted with  $\text{CH}_2\text{Cl}_2$  (3 x 6 mL). The combined organic extracts were dried and evaporated to leave a pale orange oil. Radial chromatography (4 mm  $\text{SiO}_2$ ; 0.5% MeOH in  $\text{CH}_2\text{Cl}_2$ ) facilitated the recovery of the unreacted iodopropane (3.53) (206 mg, 96%) as the first eluted compound. The second band contained methyl 2-methyl-3-oxononanoate (3.54) (80 mg, 80%) as a colourless oil, which displayed identical  $^1\text{H}$  NMR data to 3.54 prepared by Nakajima *et al.*



$^1\text{H}$  NMR (80 MHz,  $\text{CDCl}_3$ )  $\delta$  0.88 (m, 3H, H<sub>9</sub>), 1.10-1.90 (m, 3H, H<sub>5,6,7,8</sub>), 1.33 (d,  $J = 7$  Hz, 3H, H<sub>10</sub>), 2.52 (t,  $J = 7$  Hz, 2H, H<sub>4</sub>), 3.52 (q,  $J = 7$  Hz, 1H, H<sub>2</sub>), 3.72 (s, 3H, OMe).

EIMS  $m/z$  200 ( $\text{M}^+$ , 0.6%), 169 ( $\text{M}^+ - \text{OMe}$ , 1), 127 (4), 117 (5), 113 (100), 85 (29), 69 (8), 57 (18), 55 (13), 43 (97).

**1,3-Bis(*t*-butyldimethylsilyloxy)propanone (3.55)**

Redistilled dihydroxyacetone dimer (1.00 g, 11.1 mmol) was dissolved in anhydrous DMF (5 mL, 20°C) and treated with a solution of TBDMS-chloride (3.37 g, 22.4 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (25 mL). Triethylamine (2.43 g, 24.0 mmol) was then introduced, dropwise followed by DMAP (50 mg). After stirring for 17 hours at room temperature the resulting yellow suspension was washed sequentially with water (2 x 10 mL), saturated aqueous ammonium chloride solution (2 x 10 mL) and water (10 mL). The organic phase was dried and the solvent removed, leaving a yellow oil which was distilled (Kugelrohr, oven temp. 100°C / 0.25 mm Hg). After a fore-fraction containing DMF and a small amount of monoprotected product (**4.7**), the diprotected dihydroxyacetone (**3.55**) was collected as a pale yellow, viscous oil (2.90 g, 82%).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.08 (s, 12H, SiCH<sub>3</sub>) 0.91 (s, 18H, *t*-Bu), 4.40 (s, 4H, H1,3).

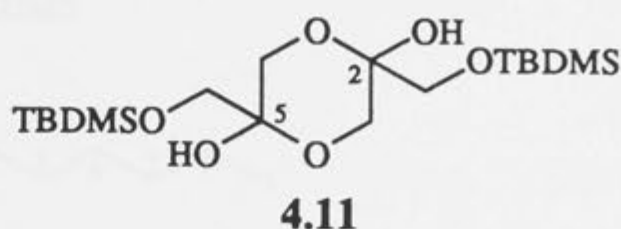
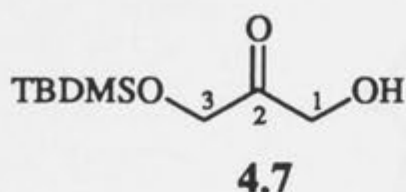
<sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ -5.7 (SiCH<sub>3</sub>), 18.1 (q, *t*-Bu), 25.6 (*t*-Bu), 66.6 (C1,3), 211.1 (C=O).

EIMS *m/z* 261 (M<sup>+</sup>-C<sub>4</sub>H<sub>9</sub>, 81%), 231 (48), 145 (13), 133 (15), 129 (18), 115 (22), 89 (100), 75 (38), 73 (93).

CIMS *m/z* 336 (M+NH<sub>4</sub><sup>+</sup>, 100%), 319 (M+H<sup>+</sup>, 92).

Analysis: Found C 56.2, H 10.8; C<sub>15</sub>H<sub>34</sub>O<sub>3</sub>Si<sub>2</sub> requires C 56.5, H 10.8.



3-(*t*-Butyldimethylsilyloxy)-1-hydroxypropanone (4.7)

Redistilled dihydroxyacetone dimer (28.0 g, 0.311 mol) was dissolved in anhydrous DMF (50 mL) and  $\text{CH}_2\text{Cl}_2$  (50 mL) before the addition of TBDMS-chloride (18.8 g, 0.125 mol) as a solid. Triethylamine (15.2 g, 0.150 mol) was then slowly introduced, causing some precipitation and warming of the mixture. Finally, DMAP (200 mg) was added and the mixture was stirred for 18 hours at room temperature under an atmosphere of argon. After pouring into water (150 mL) the organic phase was separated and the aqueous phase extracted with  $\text{CH}_2\text{Cl}_2$  (3 x 30 mL). Drying of the combined organic phases followed by removal of the solvent gave a pale yellow oil which was fractionally distilled under reduced pressure. A fore-fraction, mainly consisting of DMF was discarded before the mono-protected dihydroxyacetone (4.7) (17.4 g, 68% based on TBDMS-chloride) was collected; bp 72–76°C / 0.45 mm Hg.

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.08 (s, 6H,  $\text{SiCH}_3$ ), 0.91 (s, 9H,  $t\text{-Bu}$ ), 4.30 (s, 2H, H3), 4.49 (s, 2H, H1).

$^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ )  $\delta$  -5.7 ( $\text{SiCH}_3$ ), 18.1 (q,  $t\text{-Bu}$ ), 25.6 ( $t\text{-Bu}$ ), 66.5 (C3), 67.7 (C1), 211.1 ( $\text{C=O}$ ).

EIMS  $m/z$  205 ( $\text{M}+\text{H}^+$ , 0.4%), 159 (6), 147 (17), 129 (6), 117 (100), 89 (15), 75 (45), 73 (27), 59 (12).

CIMS  $m/z$  205 ( $\text{M}+\text{H}^+$ , 19).

Analysis: Found C 52.9, H 9.7;  $\text{C}_9\text{H}_{20}\text{O}_3\text{Si}$  requires C 52.9, H 9.9.

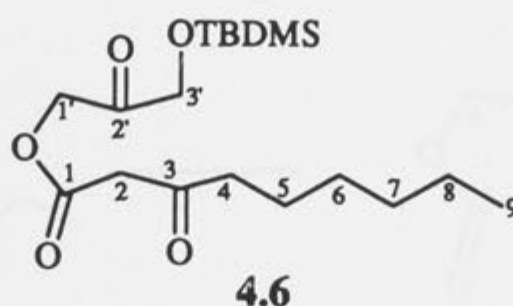
The residue after distillation was found by  $^1\text{H}$  NMR to contain the diprotected dihydroxyacetone (3.55) (*ca* 1.2 g, 3% based on TBDMS-chloride) (spectral data above).

The product (4.7) readily dimerises on storage to form some 2,5-dihydroxy-2,5-bis(*t*-butyldimethylsilyloxymethyl)-1,4-dioxane (4.11), a process which may be reversed by: i) distillation; ii) brief heating (*ca* 150°C, 5 min, under argon); or iii) chromatography on silica gel. The presence of the dimer is easily detected by NMR spectroscopy (4.11).

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.05, 0.09, 0.13 (s,  $\text{SiCH}_3$ ), 0.88, 0.89, 0.90, 0.92, 0.93 (s,  $t\text{-Bu}$ ), 3.40–4.25 (m,  $\text{CH}_2$ ).

$^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ )  $\delta$  -5.8, 18.0, 25.6, 25.7, 63.0, 63.4, 64.2, 64.4, 72.8, 74.0, 103.1, 103.57, 111.3, 111.3.



3-(*t*-Butyldimethylsilyloxy)-2-oxopropyl 3-oxononanoate (4.6)

Mono-protected dihydroxyacetone (**4.7**, 2.76 g, 13.5 mmol) in a flame-dried flask under argon, was converted into its monomeric form by brief heating (5 min) to *ca* 150°C using an oil bath. After cooling to room temperature it was dissolved in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (30 mL) before 3-oxononanoic acid (**3.33c**, 2.28 g, 13.2 mmol), DMAP (120 mg) and DMAP-hydrochloride (50 mg) were added as solids. The resulting solution was cooled to -10°C and stirred for 10 min before DCC (2.86 g, 13.8 mmol) was introduced, also in solid form. After stirring the lemon-yellow suspension at -10°C for 30 min and then at 10°C for 1 hour, reverse phase TLC analysis (RP8, 10% H<sub>2</sub>O in MeOH) detected no β-ketoacid and the mixture was then cooled to -20°C to encourage further crystallisation of the *N,N*-dicyclohexylurea. This was removed by filtration and the filter pad was washed well with cold ether (-15°C, 2 x 10 mL). The filtrate was diluted with additional ether (20 mL) and washed sequentially with dilute aqueous hydrochloric acid (2 x 15 mL), water (2 x 10 mL), and pH 7.0 phosphate buffer (20 mL). The organic layer was dried, filtered and then evaporated to an oily suspension on the rotary evaporator. Dissolution in a small volume of ether (10 mL), cooling to -20°C for 30 min and refiltration removed more urea and left the crude diketoester (**4.6**) as a clear, light-tan oil (5.72 g) when the solvent was removed. The product content of this oil was estimated as *ca* 3.8 g (*ca* 80% yield) by <sup>1</sup>H NMR integration, using an internal integration standard (diphenylmethanol). A small sample of the ester (**4.6**) was purified for spectroscopic analysis, with considerable loss, by bulb to bulb distillation in one-piece apparatus under very high vacuum (oven temp. 110°C / 0.004 mm Hg).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.10 (s, 6H, SiCH<sub>3</sub>), 0.80-0.95 (m, 12H, <sup>*t*</sup>Bu + H<sub>9</sub>), 1.20-1.40 (m, 6H, H<sub>6,7,8</sub>), 1.50-1.65 (m, 2H, H<sub>5</sub>), 2.59 (t, *J* = 7.4 Hz, 2H, H<sub>4</sub>), 3.54 (s, 2H, H<sub>2</sub>), 4.26 (s, 2H, H<sub>3'</sub>), 5.01 (s, 2H, H<sub>1'</sub>).

<sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ -5.8 (SiCH<sub>3</sub>), 13.9 (C<sub>9</sub>), 18.1 (q, <sup>*t*</sup>Bu), 22.4 (C<sub>8</sub>), 23.3 (C<sub>5</sub>), 25.6 (<sup>*t*</sup>Bu), 28.6 (C<sub>6</sub>), 31.4 (C<sub>7</sub>), 42.9 (C<sub>4</sub>), 48.7 (C<sub>2</sub>), 67.4 (C<sub>3'</sub>)\*, 68.1 (C<sub>1'</sub>)\*, 166.5 (C<sub>1</sub>), 202.4 (C<sub>2'</sub>)\*, 203.3 (C<sub>3</sub>)\*.

EIMS *m/z* 301 (M<sup>+</sup>-57, 0.2%), (187 (0.4), 171 (3), 155 (3), 129 (100), 113 (20), 85 (12), 75 (26), 73 (48), 69 (11), 59 (30), 58 (26), 57 (44).

HRMS: Found (M<sup>+</sup>-C<sub>4</sub>H<sub>9</sub>) 301.1470; C<sub>14</sub>H<sub>25</sub>O<sub>5</sub> requires 301.1472.



Cyclisation of 3-(*t*-Butyldimethylsilyloxy)-2-oxopropyl 3-oxononanoate (4.6)

## METHOD A: USING SILICA GEL

Crude diketoester (4.6) (250 mg [*ca* 80% pure], *ca* 0.56 mmol) as prepared above was subjected to radial chromatography using a rotor coated with a 2 mm layer of Merck 60 PF<sub>254</sub> silica gel. Before chromatography, the rotor was activated by drying for 24 hours at 70°C. Progress of the chromatography was monitored with a short wavelength UV lamp. Hexane (20%) in CH<sub>2</sub>Cl<sub>2</sub> was used as eluent until the compound band had traversed approximately one quarter of the plate at which time the eluent was changed to CH<sub>2</sub>Cl<sub>2</sub> containing a small amount of MeOH (0.1%). The major UV-absorbing band eluted was the 2-heptanoyl-3-(*t*-butyldimethylsilyloxymethyl)but-2-enolide (4.8) which appeared as a pale yellow oil (152 mg, 64%) based on 3-oxononanoic acid (3.33c).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.10 (s, 6H, SiCH<sub>3</sub>), 0.80-1.00 (m, 12H, <sup>*t*</sup>Bu + H<sub>9</sub>), 1.20-1.40 (m, 6H, H<sub>6,7,8</sub>), 1.55-1.67 (m, 2H, H<sub>5</sub>), 2.97 (t, *J* = 7.3 Hz, 2H, H<sub>7</sub>), 4.95 (s, 2H, H<sub>5</sub>)\*, 5.03 (s, 2H, H<sub>4</sub>)\*.

<sup>13</sup>C NMR (75.5 MHz, CDCl<sub>3</sub>) δ -5.7 (SiCH<sub>3</sub>), 14.0 (C<sub>12</sub>), 18.1 (q, <sup>*t*</sup>Bu), 22.4 (C<sub>11</sub>)\*, 23.1 (C<sub>8</sub>)\*, 25.6 (<sup>*t*</sup>Bu), 28.7 (C<sub>9</sub>), 31.6 (C<sub>10</sub>), 41.6 (C<sub>7</sub>), 61.8 (C<sub>5</sub>), 70.2 (C<sub>4</sub>), 122.4 (C<sub>2</sub>), 170.6 (C<sub>3</sub>), 181.1 (C<sub>1</sub>), 197.1 (C<sub>6</sub>).

IR ν<sub>max</sub> (CCl<sub>4</sub>) 2930 s, 2860 m, 1775 s (lactone C=O), 1688 m (ketone C=O), 1630 m (C=C), 1465 w, 1260 m, 1110 m, 1045 m, 1005 m cm<sup>-1</sup>.

UV λ<sub>max</sub> (MeOH) 236 nm (ε 8100), 314 nm (ε 2200); (0.004 M NaOH in MeOH) 218 nm (ε 8600), 314 nm (11600).

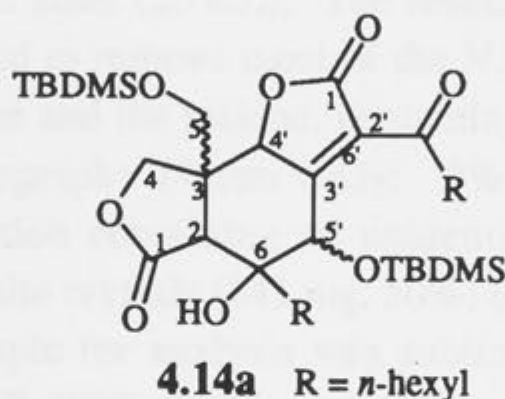
EIMS *m/z* 340 (M<sup>+</sup>, 3%), 283 (M<sup>+</sup>-C<sub>4</sub>H<sub>9</sub>, 50), 255 (9), 253 (14), 228 (7), 213 (6), 199 (19), 183 (7), 171 (12), 169 (12), 153 (8), 129 (7), 113 (19), 85 (14), 75 (100), 73 (68), 43 (75).

HRMS: Found (M<sup>+</sup>) 340.2070; C<sub>11</sub>H<sub>15</sub>O<sub>7</sub> requires 340.2070.



## METHOD B: USING SODIUM HYDRIDE

Crude diketoester (**4.6**) (500 mg [*ca* 80% pure], *ca* 1.1 mmol) as prepared above was dissolved in anhydrous THF (100 mL) and cooled to 0°C. Sodium hydride (50% dispersion in oil, 67 mg, 1.4 mmol) was added and the suspension stirred for 40 min after which time reverse phase TLC (RP8, 10% H<sub>2</sub>O in MeOH) showed no remaining ester (**4.6**, *R<sub>f</sub>* 0.38). The reaction was quenched by the addition of pH 5.0 phosphate buffer (15 mL) and water (20 mL) before approximately half of the THF was removed by rotary evaporation (< 20°C). The mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 15 mL) and the extracts were washed with water (20 mL), dried and evaporated to leave an oil which was subjected to rapid radial chromatography (4 mm SiO<sub>2</sub>; 0.1-1% MeOH in CH<sub>2</sub>Cl<sub>2</sub>). The first eluted band contained the butenolide (**4.8**) (304 mg, 65% based on 3-oxononanoic acid (**3.33c**) (see spectroscopic data above). A minor band was eluted next which contained the tricyclic dimer (**4.14a**) (28 mg, 3% based on 3-oxononanoic acid) as fine colourless prisms (mp 130-131°C from pentane).



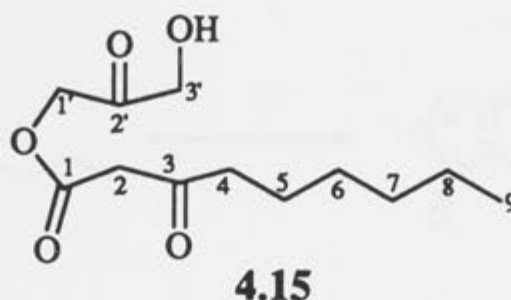
<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ -0.02, 0.09, 0.10, 0.12 (4 x s, 12H, 4 x SiCH<sub>3</sub>), 0.75-1.00 (m, 24H, 2 x CH<sub>3</sub> + 2 x <sup>t</sup>Bu), 1.15-1.70 (m, 20H), 1.88 (m, 1H, H7b), 2.25 (m, 1H, H7a), 2.70 (s, 1H, H2), 2.97 (m, 2H, H7'), 3.64 (d, *J*<sub>5a,5b</sub> = 10.3 Hz, 1H, H5b), 3.81 (d, 1H, *J*<sub>5a,5b</sub> = 10.3 Hz, H5a), 3.81 (d, *J*<sub>4a,4b</sub> = 9.0 Hz, 1H, H4b), 3.87 (d, 1H, *J*<sub>4a,4b</sub> = 9.0 Hz, H4a), 5.36 (s, 1H, H5'), 5.41 (s, 1H, H4').

EIMS *m/z* 680 (M<sup>+</sup>, 4%), 625 (15), 624 (38), 623 (85), 605 (21), 339 (8), 113 (12), 89 (25), 75 (52), 73 (100), 57 (17), 55 (15).

CIMS *m/z* 698 (M+NH<sub>4</sub><sup>+</sup>, 20%), 681 (M+H<sup>+</sup>, 58), 663 (31), 623 (M-C<sub>4</sub>H<sub>9</sub>, 100), 605 (34), 566 (40), 549 (60), 533 (43), 341 (51), 283 (23).

HRMS: Found (M<sup>+</sup>) 680.4139; C<sub>36</sub>H<sub>64</sub>O<sub>8</sub>Si requires 680.4140.



**3-Hydroxy-2-oxopropyl 3-oxononanoate (4.15)**

Freshly distilled dihydroxyacetone (378 mg, 4.2 mmol), 3-oxononanoic acid (**3.33c**) (200 mg, 1.16 mmol), DMAP (10 mg) and DMAP hydrochloride (10 mg) were dissolved, with warming, in anhydrous THF (8 mL). The solution was cooled to 0°C before DCC (260 mg, 1.26 mmol) was added and the temperature allowed to rise to 20°C. After 2.5 hours pH 3.5 phosphate buffer (10 mL) was added and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (4 x 10 mL). The combined organic extracts were washed with water (10 mL), dried and evaporated to leave a greenish yellow semi-solid residue which was taken up in warm ether (20 mL). The resulting suspension was cooled to room temperature and filtered to remove most of the *N,N*-dicyclohexylurea. Solvent was removed from the filtrate and the residue, containing largely the ester (**4.15**), was purified by radial chromatography (4 mm SiO<sub>2</sub>; 5% MeOH in CH<sub>2</sub>Cl<sub>2</sub>). After collecting a small fore-fraction containing an unidentified mixture, the β-ketoester (**4.15**) was isolated as off-white crystals (141 mg, 50%) (mp 68-70°C, colourless plates from ether-hexane). A sample for analysis was sublimed under very high vacuum (70°C / 0.005 mm Hg); mp 67-70°C.

<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) δ 0.89 (t, *J* = 7 Hz, H<sub>9</sub>), 1.15-1.45 (m, 6H, H<sub>6,7,8</sub>), 1.55-1.75 (m, 2H, H<sub>5</sub>), 2.58 (t, *J* = 7.3 Hz, H<sub>4</sub>), 3.58 (s, 2H, H<sub>2</sub>), 4.40 (s, 2H, H<sub>3'</sub>), 4.84 (s, 2H, H<sub>1'</sub>).

<sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ 14.0 (C<sub>9</sub>), 22.4 (C<sub>8</sub>), 23.4 (C<sub>5</sub>), 28.6 (C<sub>6</sub>), 31.5 (C<sub>7</sub>), 43.1 (C<sub>4</sub>), 48.5 (C<sub>2</sub>), 66.3 (C<sub>3'</sub>)\*, 66.6 (C<sub>1'</sub>)\*, 166.5 (C<sub>1</sub>), 202.3 (C<sub>2'</sub>)\*, 203.8 (C<sub>3</sub>)\*.

EIMS *m/z* 213 (M<sup>+</sup>-CH<sub>2</sub>OH, 0.4%), 197 (5), 174 (3), 155 (6), 113 (48), 85 (18), 84 (30), 69 (17), 58 (16), 57 (14), 55 (16), 43 (100).

CIMS *m/z* 262 (M+NH<sub>4</sub><sup>+</sup>, 100%), 245 (M+H<sup>+</sup>, 9).

Analysis: Found C 59.2, H 8.5; C<sub>12</sub>H<sub>20</sub>O<sub>5</sub> requires C 59.0, H 8.3.



3-(*t*-Butyldimethylsilyloxymethyl)-2-heptanoylbutanolide (4.27)

The butenolide (**4.8**) (40.2 mg, 0.115 mmol) in MeOH (10 mL) was hydrogenated over 10% palladium-on-charcoal for 40 minutes, at which time 1 equivalent of hydrogen had been absorbed and TLC analysis ( $\text{CH}_2\text{Cl}_2$ ; **4.8**  $R_f$  = 0.82, **4.27**  $R_f$  = 0.70) indicated no starting material. The catalyst was removed by filtration through a 5 mm bed of silica-gel (Merck 60 PF<sub>254</sub>) and the filtrate was evaporated, leaving the crude saturated lactone product (**4.27**) as a colourless oil. Radial chromatography (1 mm SiO<sub>2</sub>; 0-5% MeOH in  $\text{CH}_2\text{Cl}_2$ ) furnished 34.5 mg (88%) of purified material.

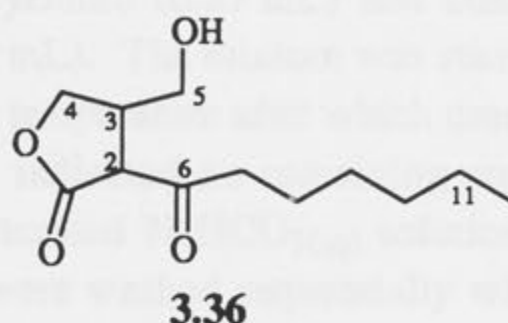
<sup>1</sup>H NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.04 (s, 6H, SiCH<sub>3</sub>), 0.88 (m, 12H, <sup>*t*</sup>Bu+H12), 1.20-1.40 (m, 6H, H9,10,11), 1.50-1.70 (m, 2H, H8), 2.61 (dt,  $J_{7a,7b}$  = 17.8 Hz,  $J_{7,8}$  = 7.3 Hz, 1H, H7b), 2.94 (dt, 1H, H7a), 3.18 (m, 1H, H3), 3.62 (d,  $J$  = 4.5 Hz, 2H, H5), 3.64 (d,  $J_{2,3}$  = 6.8 Hz, 1H, H2), 4.12 (dd,  $J_{4a,4b}$  = 8.9 Hz,  $J_{4b,3}$  = 6.5 Hz, 1H, H4b), 4.39 (dd,  $J_{4a,4b}$  = 8.9 Hz,  $J_{4a,3}$  = 8.0 Hz, 1H, H4a).

<sup>13</sup>C NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  -5.6 (SiCH<sub>3</sub>), 14.0 (C12), 18.1 (q, <sup>*t*</sup>Bu), 22.4 (C11), 23.2 (C8), 25.7 (<sup>*t*</sup>Bu), 28.6 (C9), 31.5 (C10), 39.3 (C3), 42.5 (C7), 54.7 (C2), 61.9 (C5), 69.1 (C4), 172.4 (C1), 202.7 (C6).

EIMS  $m/z$  327 ( $\text{M}^+ - \text{CH}_3$ , 2%), 285 ( $\text{M}^+ - \text{C}_4\text{H}_9$ , 50), 167 (5), 201 (13), 187 (9), 173 (7), 127 (6), 113 ( $\text{C}_6\text{H}_{13}\text{CO}^+$ , 61), 85 (30), 75 (62), 73 (38), 69 (10), 59 (11), 57 (22), 55 (24), 43 (100).

HRMS: Found ( $\text{M}^+ - \text{C}_4\text{H}_9$ ) 285.1523;  $\text{C}_{14}\text{H}_{25}\text{O}_4\text{Si}$  requires 285.1522.



**3-Hydroxymethyl-2-heptanoylbutanolide (3.36)****[i.e. 11-demethyl A-factor]****METHOD A: DESILYLATION WITH GLACIAL ACETIC ACID : THF : WATER (3 : 3 : 1)**

The silyl butanolide (**4.27**) (50 mg, 0.146 mmol) was dissolved in a mixture of glacial acetic acid : THF : water (2.5 mL, 3 : 3 : 1) and stirred for 48 hours at room temperature. The bulk of the solvent mixture was removed on the rotary evaporator and the remaining traces were evaporated using an oil pump. The oily residue was separated into two compounds using radial chromatography (1 mm SiO<sub>2</sub>; 5% MeOH in CH<sub>2</sub>Cl<sub>2</sub>). The first eluted compound was unreacted silyl butanolide (**4.27**) (10 mg, 20%) followed by 11-demethyl A-factor (**3.36**) (19 mg, 57%) as a colourless oil, which was immediately analysed by NMR spectroscopy.

<sup>1</sup>H NMR (300 MHz, acid-free CDCl<sub>3</sub>) δ 0.88 (t, *J* = 7 Hz, CH<sub>3</sub>), 1.15-1.45 (m, 6H, H<sub>9,10,11</sub>), 1.50-1.70 (m, 2H, H<sub>8</sub>), 2.64 (dt, *J*<sub>7a,7b</sub> = 17.9 Hz, *J*<sub>7b,8</sub> = 7.2 Hz, 1H, H<sub>7b</sub>), 2.99 (dt, *J*<sub>7a,7b</sub> = 17.9 Hz, *J*<sub>7a,8</sub> = 7.2 Hz, 1H, H<sub>7a</sub>), 3.25 (m, 1H, H<sub>3</sub>), 3.67 (d, *J*<sub>2,3</sub> = 6.9 Hz, 1H, H<sub>2</sub>), 3.67 (dd, *J*<sub>5b,3</sub> = 5.4 Hz, *J*<sub>5b,5a</sub> = 10.5 Hz, 1H, H<sub>5b</sub>), 3.74 (dd, *J*<sub>5a,3</sub> = 5.6 Hz, *J*<sub>5a,5b</sub> = 10.5 Hz, 1H, H<sub>5a</sub>), 4.14 (dd, *J*<sub>4b,3</sub> = 6.6 Hz, *J*<sub>4a,4b</sub> = 9.0 Hz, 1H, H<sub>4b</sub>), 4.44 (dd, *J*<sub>4a,3</sub> = 8.2 Hz, *J*<sub>4a,4b</sub> = 9.0 Hz, 1H, H<sub>4a</sub>), 11.20 (s, enol OH) [*ca* 4% enol content]. Additional complex minor peaks are attributed to small amounts (< 20%) of the hemiacetal tautomers (**5.22a** and **5.22b**) described below.

<sup>13</sup>C NMR (75 MHz, acid-free CDCl<sub>3</sub>) δ 14.0 (C<sub>12</sub>), 22.4 (C<sub>11</sub>), 23.5 (C<sub>8</sub>), 28.5 (C<sub>9</sub>), 31.5 (C<sub>10</sub>), 39.1 (C<sub>3</sub>), 42.5 (C<sub>7</sub>), 54.9 (C<sub>2</sub>), 61.8 (C<sub>5</sub>), 68.9 (C<sub>4</sub>), 174.7 (C<sub>1</sub>), 202.8 (C<sub>6</sub>).

FTIR (CCl<sub>4</sub>)  $\nu_{\max}$  3603 m, 3483 w, 2958 s, 2958 s, 1785 s (lactone C=O), 1720 m (ketone C=O), 1647 w, 1467 m, 1380 m, 1177 s, 1044 s cm<sup>-1</sup>.

EIMS *m/z* 228 (M<sup>+</sup>, 0.5%), 210 (M<sup>+</sup>-H<sub>2</sub>O, 0.8), 197 (M<sup>+</sup>-CH<sub>2</sub>OH, 16), 171 (M<sup>+</sup>-C<sub>4</sub>H<sub>9</sub>, 3), 158 (M<sup>+</sup>-70 McLafferty rearr., 5), 143 (M<sup>+</sup>-C<sub>6</sub>H<sub>13</sub>, 24), 127 (11), 116 (13), 113 (68), 102 (15), 85 (100), 69 (15), 57 (39), 55 (55).

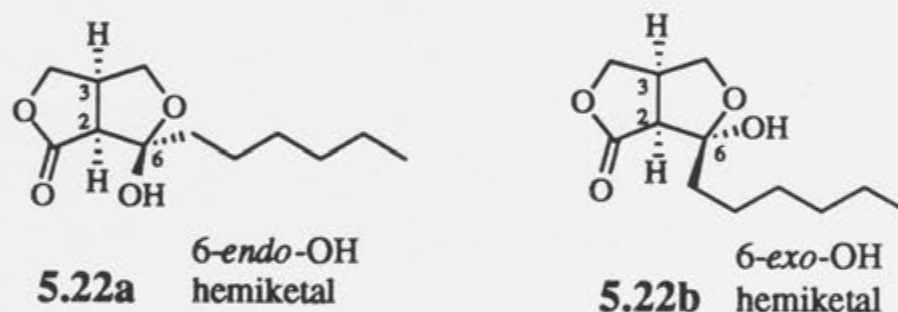
HRMS: Found (M<sup>+</sup>) 228.1361; C<sub>12</sub>H<sub>20</sub>O<sub>4</sub> requires 228.1362.

The above <sup>1</sup>H NMR, IR and EIMS data are consistent with that previously reported for this compound.<sup>35</sup>



METHOD B: DESILYLATION WITH  $\text{HF}_{\text{aq}}$  / PYRIDINE /  $\text{CH}_3\text{CN}$ 

The silyl butanolide (**4.27**) (192 mg, 0.56 mmol) in  $\text{CH}_3\text{CN}$  (20 mL) at  $0^\circ\text{C}$  was treated sequentially with pyridine (0.27 mL) and concentrated aqueous hydrogen fluoride solution (50%, 1.35 mL). The mixture was stirred for 30 minutes at  $0^\circ\text{C}$  then for a further 4 hours at room temperature after which time TLC (1% MeOH in  $\text{CH}_2\text{Cl}_2$ , **4.27**  $R_f$  0.80; **3.36**  $R_f$  0.10) indicated no remaining starting material. The product mixture was poured into saturated  $\text{NaHCO}_3(\text{aq})$  solution and extracted with  $\text{CH}_2\text{Cl}_2$  (3 x 10 mL). The extracts were washed sequentially with water (10 mL) and pH 7.0 phosphate buffer (10 mL), then were dried and evaporated under high-vacuum to yield a yellow oil. Purification by radial chromatography (2 mm  $\text{SiO}_2$ ; 10-40% EtOAc in hexane) gave 11-demethyl A-factor (**3.36**), (115 mg, 90%) as a colourless oil which partially solidified on cooling.  $^1\text{H}$  and  $^{13}\text{C}$  NMR analyses performed immediately following chromatography gave identical data to that described for (**3.36**) above (Method A). However, after careful recrystallisation from cold ( $-15^\circ\text{C}$ )  $\text{CCl}_4$  / hexane, the bicyclic hemiacetal tautomer (**5.22a**) was isolated as colourless platelets (mp  $60-65^\circ\text{C}$ ). The *endo* stereochemistry of the tertiary hydroxyl group was assigned by NMR comparison with the equivalent 11-methyl analogue (**5.12a**) whose structure was established by X-ray diffraction.

Data for **5.22a**:

$^1\text{H}$  NMR (300 MHz, acid-free  $\text{CDCl}_3$ )  $\delta$  0.89 (t,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ ), 1.25-1.42 (m, 6H, H9,10,11), 1.45-1.57 (m, 2H, H8), 1.89 ('dd',  $J = 6.7$  Hz,  $J = 10.0$  Hz, 2H, H7), 2.79 (d,  $J_{2,\text{OH}} = 1.1$  Hz, 1H, OH), 3.14 (dd,  $J_{2,3} = 10.5$  Hz,  $J_{2,\text{OH}} = 1.1$  Hz, 1H, H2), 3.43 (m, 1H, H3), 4.02 (dd,  $J_{5b,5a} = 9.0$  Hz,  $J_{5b,3} = 5.1$  Hz, 1H, H5a), 4.21 (dd,  $J_{5a,3} = 8.2$  Hz,  $J_{5b,5a} = 9.0$  Hz, 1H, H5a), 4.27 (dd,  $J_{4b,4a} = 9.0$  Hz,  $J_{4b,3} = 6.0$  Hz, H, H4b), 4.50 (t,  $J_{4a,3} = 9.0$  Hz,  $J_{4b,4a} = 9.0$  Hz, 1H, H4a).

$^{13}\text{C}$  NMR (75 MHz, acid-free  $\text{CDCl}_3$ )  $\delta$  14.0 (C12), 22.5 (C11), 23.4 (C8), 29.2 (C9), 31.6 (C10), 39.1 (C3), 40.9 (C7), 53.7 (C2), 71.9 (C5)\*, 72.3 (C4)\*, 106.7 (C6), 174.5 (C1).

FTIR (KBr disc)  $\nu_{\text{max}}$  3397 s (OH), 2930 s, 1760 s, 1719 sh, 1466 m, 1394 m, 1190 s, 1029 s, 948 m, 908 m, 611  $\text{cm}^{-1}$ .

EIMS  $m/z$  228 ( $\text{M}^+$ , 0.5%), 210 ( $\text{M}^+ - \text{H}_2\text{O}$ , 0.8), 197 ( $\text{M}^+ - \text{CH}_2\text{OH}$ , 16), 171 ( $\text{M}^+ - \text{C}_4\text{H}_9$ , 3), 158 ( $\text{M}^+ - 70$  McLafferty rearr., 5), 143 ( $\text{M}^+ - \text{C}_6\text{H}_{13}$ , 24), 127 (11), 116 (13), 113 (68), 102 (15), 85 (100), 69 (15), 57 (39), 55 (55).

HRMS: Found ( $\text{M}^+$ ) 228.1361;  $\text{C}_{12}\text{H}_{20}\text{O}_4$  requires 228.1362.

The  $^1\text{H}$  and  $^{13}\text{C}$  NMR sample solutions were allowed to stand for 5 hours before being re-run. The new spectra indicated the presence of *ca* 65% of the bicyclic



hemiacetal product described above (**5.22a**), *ca* 10% of 2,3-*trans* 11-demethyl A-factor (**3.36**, see data above) and *ca* 25% of the alternate, *exo*-hydroxy diastereomer of the bicyclic hemiacetal (**5.22b**).

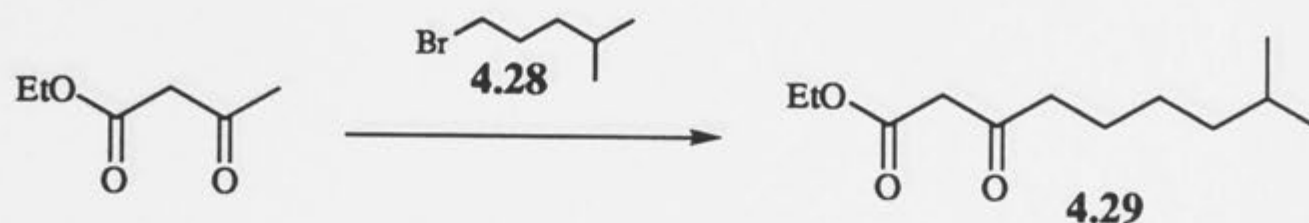
Data for **5.22b**:

$^1\text{H}$  NMR (300 MHz, acid-free  $\text{CDCl}_3$ )  $\delta$  0.88 (t,  $J = 7$  Hz, 3H,  $\text{CH}_3$ ), 1.25-1.42 (m, 6H, H9,10,11), 1.45-1.60 (m, 2H, H8), 1.78 (ddd,  $J_{7a,7b} = 13.9$  Hz,  $J = 11.5$  Hz,  $J = 4.8$  Hz, 1H, H7b), 2.06 (ddd,  $J_{7a,7b} = 13.9$  Hz,  $J = 11.6$  Hz,  $J = 4.6$  Hz, 1H, H7a), 2.31 (s, 1H, OH), 3.20 (d,  $J_{2,3} = 9.3$  Hz, 1H, H2), 3.40 (m, 1H, H3), 3.88 (dd,  $J_{5a,5b} = 9.3$  Hz,  $J_{5b,3} = 1.7$  Hz, 1H, H5b), 4.11 (dd,  $J_{5a,5b} = 9.3$  Hz,  $J_{5a,3} = 4.8$  Hz, 1H, H5a), 4.27 (dd,  $J_{4a,4b} = 9.2$  Hz,  $J_{4a,3} = 5.9$  Hz, 1H, H4b), 4.49 (dd,  $J_{4a,4b} = 9.2$  Hz,  $J_{4a,3} = 9.1$  Hz, 1H, H4a).

$^{13}\text{C}$  NMR (75 MHz, acid-free  $\text{CDCl}_3$ )  $\delta$  14.0 (C12) 22.5 (C11), 23.5 (C8), 29.3 (C9), 31.6 (C10), 36.7 (C3), 39.7 (C7), 53.6 (C2), 71.4 (C5)\*, 72.5 (C4)\*, 107.5 (C6), 174.5 (C1).

After 1 week at room temperature the four tautomeric components reached an equilibrium ratio of 68 : 14 : 14 : 4, (**3.36** : **5.22a** : **5.22b** : **3.36** enol) as determined by  $^1\text{H}$  NMR integration.

## Ethyl 8-methyl-3-oxononanoate (4.29)



Ethyl acetoacetate (13.0 g, 0.10 mol) in anhydrous THF (10 mL) was converted to its dianion prior to alkylation by slow addition to a cold ( $-5^{\circ}\text{C}$ ) suspension of sodium hydride (50% oil dispersion, 5.3 g, 0.11 mol) in THF (250 mL), followed by the cautious addition of *n*-butyllithium solution (1.54 M in hexane, 66 mL, 0.10 mol) in a manner similar to that of Huckin and Weiler.<sup>118</sup> Alkylation with 1-bromo-4-methylpentane (18.2 g, 0.11 mol) followed by an aqueous work-up produced an oil which was fractionally distilled at reduced pressure. The first fraction contained unreacted 1-bromo-4-methylpentane (4.28) (5.1 g, bp  $55^{\circ}\text{C}$  / 27 mm Hg). The pressure was further reduced to collect the title ester (4.29) (13.3 g, 62%) as a colourless oil (bp  $86\text{--}88^{\circ}\text{C}$  / 0.1 mm Hg).

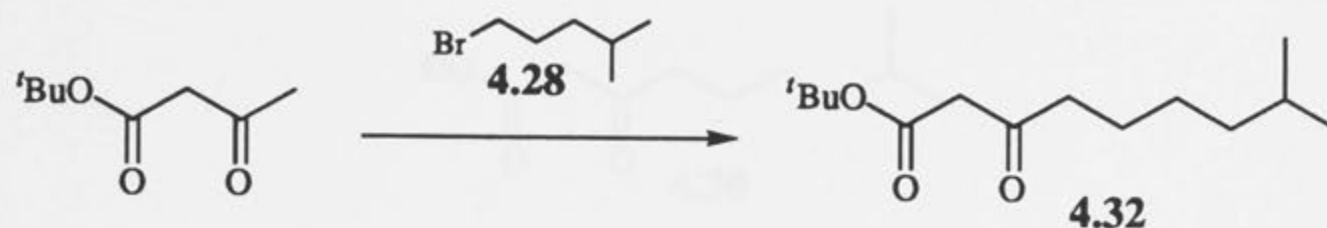
$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ )  $\delta$  0.86 (d,  $J = 6.5$  Hz, 6H, 2 x  $\text{CH}_3$ ), 1.10–1.40 (m, 7H, ethyl  $\text{CH}_3$ +H6,7), 1.45–1.67 (m, 3H, H5,8), 2.2 (t,  $J = 7.5$  Hz, enol H4), 2.54 (t,  $J = 7.5$  Hz, 2H, H4), 3.43 (s, 2H, H2), 4.20 (q,  $J = 7.2$  Hz, ethyl  $\text{CH}_2$ ), 4.98 (s, enol H2), 12.1 (s, enol OH), [ca 11% enol].

$^{13}\text{C}$  NMR (50.3 MHz,  $\text{CDCl}_3$ )  $\delta$  14.0 (ethyl  $\text{CH}_3$ ), 14.2 (enol ethyl  $\text{CH}_3$ ), 22.5 (2x $\text{CH}_3$ ), 23.6 (C5), 26.7 (C6), 27.7 (C8), 34.9 (enol), 38.5 (C7), 43.0 (C4), 49.2 (C2), 59.8 (enol ethyl  $\text{CH}_2$ ), 61.2 (ethyl  $\text{CH}_2$ ), 88.8 (enol C2), 167.2 (C1), 172. (enol C1), 178.9 (enol C3), 202.9 (C3).

EIMS  $m/z$  214 ( $\text{M}^+$ , 3%), 196 ( $\text{M}^+ - \text{H}_2\text{O}$ , 2), 169 (3), 143 (17), 130 (100), 127 (29.6), 115 (16), 109 (59), 102 (12), 97 (17), 88 (46), 84 (59), 82 (48), 57 (81), 55 (52), 43 (91).

Analysis: Found C 67.6, H 10.5;  $\text{C}_{12}\text{H}_{22}\text{O}_3$  requires C 67.3, H 10.4.



***t*-Butyl 8-methyl-3-oxononanoate (4.32)**

A solution of *t*-butyl acetoacetate (5.7 g, 36 mmol) was converted into its dianion by the method of Huckin and Weiler<sup>118</sup> and then  $\gamma$ -alkylated with 1-bromo-4-methylpentane (5.4 g, 32.7 mmol). Fractional distillation of the crude product gave the  $\beta$ -ketoester (**4.32**), (65%, bp 72–76°C / 0.1 mm Hg) in a 75% yield based on 1-bromo-methylpentane (**4.28**).

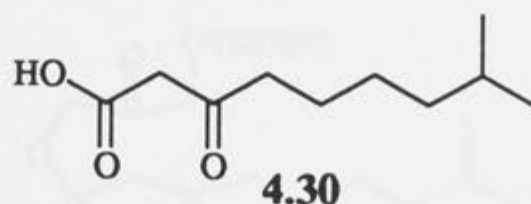
<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.83 (d,  $J$  = 6.6 Hz, 6H, 2 x CH<sub>3</sub>), 1.08–1.20 (m, 2H, H7), 1.20–1.33 (m, 2H, H6), 1.45 (s, 9H, *t*-Bu), 1.45–1.62 (m, 3H, H5,8), 2.50 (t,  $J$  = 7.4 Hz, 2H, H4), 3.32 (s, 2H, H2) [*ca* 7% enol].

<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  22.1 (2 x CH<sub>3</sub>), 23.2 (C5), 26.3 (C6), 27.3 (C8), 27.4 (*t*-Bu), 28.3 (C7), 42.4 (C4), 50.2 (C2), 81.3 (*t*-Bu quat.), 166.5 (C1), 203.3 (C3).

EIMS  $m/z$  186 (M<sup>+</sup>-C<sub>4</sub>H<sub>8</sub>, 4%), 169 (7), 168 (3), 144 (3), 127 (19), 109 (31), 102 (41), 97 (5), 84 (16), 82 (16), 57 (100), 43 (39).

Analysis: Found C 69.6, H 11.0; C<sub>14</sub>H<sub>26</sub>O<sub>3</sub> requires C 69.4, H 10.8.



**8-Methyl-3-oxononanoic acid (4.30)****METHOD A: FROM THE ETHYL ESTER USING ACETIC ACID / HYDROCHLORIC ACID**

Ethyl 8-methyl-3-oxononanoate (**4.29**) (10.0 g) was hydrolysed with a mixture of glacial acetic acid (150 mL) and concentrated hydrochloric acid (85 mL) as previously described for the preparation of 3-oxononanoic acid (**3.33c**).<sup>120</sup> The reaction mixture was evaporated under high vacuum (rotary evaporator, < 1 mm Hg, 25°C). Traces of water were azeotropically removed by the addition and evaporation of benzene (100 mL). After further drying over molecular sieves in a vacuum desiccator (24 hours, 4°C), the crude crystalline acid was recrystallised from cold pentane (-15°C). Collection by filtration of the  $\beta$ -ketoacid (**4.30**) as fine colourless needles (3.5 g, 40%, mp 44-46°C dec.) and subsequent vacuum drying was carried out at 0°C (cold room) to avoid melting of the damp crystals. The mother liquor could not be induced to crystallise further but <sup>1</sup>H NMR analysis indicated the presence of *ca* 1.7 g (20%) of the  $\beta$ -ketoacid (**4.30**).

<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>)  $\delta$  0.87 (d, *J* = 6.6 Hz, 6H, 2 x CH<sub>3</sub>), 1.10-1.40 (m, 4H, H<sub>6,7</sub>), 1.42-1.70 (m, 3H, H<sub>5,8</sub>), 2.24 (t, *J* = 6.8 Hz, enol H<sub>4</sub>), 2.57 (t, *J* = 6.8 Hz, 2H, H<sub>4</sub>), 3.53 (s, 2H, H<sub>2</sub>), 5.02 (s, enol H<sub>2</sub>), 11.87 (s, enol OH), [15% enol tautomer].

<sup>13</sup>C NMR (50.3 MHz, CDCl<sub>3</sub>)  $\delta$  22.5 (2 x CH<sub>3</sub>), 23.6 (C<sub>5</sub>), 26.4 (enol), 26.7 (C<sub>6</sub>), 26.8 (enol), 35.3 (enol), 38.6 (C<sub>7</sub>), 43.2 (C<sub>4</sub>), 48.2 (C<sub>2</sub>), 88.2 (enol C<sub>2</sub>), 172.3 (C<sub>1</sub>), 176.9 (enol C<sub>1</sub>)\*, 142.1 (enol C<sub>3</sub>)\*, 203.8 (C<sub>3</sub>).

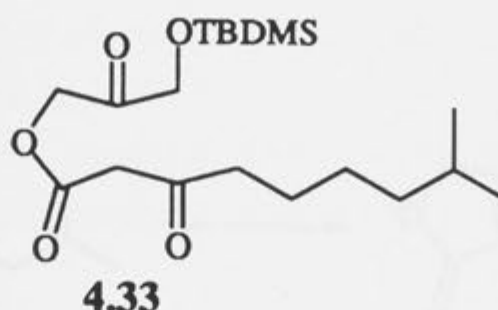
EIMS *m/z* 186 (M<sup>+</sup>, 1%), 168 (M<sup>+</sup>-H<sub>2</sub>O, 1), 153 (1), 142 (2), 115 (7), 109 (15), 102 (40), 87 (16), 84 (47), 82 (55), 71 (39), 58 (100), 57 (45), 55 (43), 43 (90).

Analysis: Found C 64.3, H 10.0; C<sub>10</sub>H<sub>18</sub>O<sub>3</sub> requires C 64.5, H 9.7.

**METHOD B: FROM THE *t*-BUTYL ESTER USING TRIFLUOROACETIC ACID**

*t*-Butyl 8-methyl-3-oxononanoate (**4.32**) was converted to the corresponding  $\beta$ -ketoacid (**4.30**) with trifluoroacetic acid as described above for the preparation of 3-oxononanoic acid (**3.33c**, Method B). Recrystallisation of the crude product from pentane (-15°C) gave the acid (**4.30**) as fine colourless needles (80%) in two crops, mp 43-45°C. NMR data was identical to that reported above (Method A). Stored at 4°C, this product has been stable towards decarboxylation for over one year.



**3-(*t*-Butyldimethylsilyloxy)-2-oxopropyl 8-methyl-3-oxononanoate (4.33)**

The title  $\beta$ -ketoester (**4.33**) was prepared from 8-methyl-3-oxononanoic acid (**4.30**), 1.0 g, 5.37 mmol) and 1-(*t*-butyldimethylsilyloxy)-3-hydroxypropane (**4.7**), (1.40 g, 6.85 mmol) by the method previously described for the synthesis of  $\beta$ -ketoester (**4.6**). The crude ester (**4.33**) was isolated as a light brown oil (2.4 g) and was found by  $^1\text{H}$  NMR analysis (using diphenylmethanol as an internal standard) to be approximately 66% pure (*ca* 1.6 g, *ca* 80% yield). The remainder consisted largely of the mono-protected dihydroxyacetone (**4.7**) and its dimer (**4.11**) as well as some *N,N*-dicyclohexylurea. A small sample of **4.33** for spectral analysis was purified by Kugelrohr distillation in a one-piece apparatus (oven temp 110°C / 0.004 mm Hg).

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.08 (s, 6H, 2 x  $\text{SiCH}_3$ ), 0.84 (d,  $J = 6.6$  Hz, 6H, 2 x  $\text{CH}_3$ ), 0.91 (s, 9H, *t*-Bu), 1.08-1.20 (m, 2H, H7), 1.20-1.35 (m, 2H, H6), 1.40-1.62 (m, 3H, H5+H8), 2.59 (t,  $J = 6.8$  Hz, 2H, H4), 3.54 (s, 2H, H2), 4.26 (s, 2H, H3'), 5.01 (s, 2H, H1').

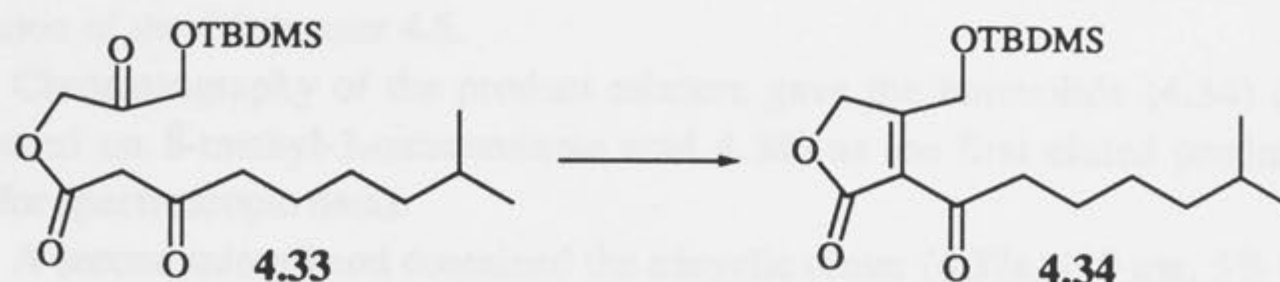
$^{13}\text{C}$  NMR (50.3 MHz,  $\text{CDCl}_3$ )  $\delta$  18.1 (quat. *t*-Bu), 22.5 (2 x  $\text{CH}_3$ ), 23.6 (C5), 25.7 (*t*-Bu), 26.7 (C6), 27.7 (C8), 38.6 (C7), 42.9 (C4), 48.8 (C2), 67.5 (C3')\*, 68.1 (C1')\*, 166.5 (C1), 202.5 (C2'), 203.4 (C3).

EIMS  $m/z$  315 ( $\text{M}^+ - \text{C}_4\text{H}_9$ , 1%), 187 (1), 171 (4), 169 (4), 147 (3), 129 (100), 127 (8), 117 (10), 109 (15), 75 (11), 73 (24), 57 (26), 56 (18), 55 (15), 43 (33).

HRMS: Found ( $\text{M}^+ - \text{C}_4\text{H}_9$ ) 315.1625;  $\text{C}_{15}\text{H}_{27}\text{O}_5\text{Si}$  requires 315.1628.



**Cyclisation of 3-(*t*-Butyldimethylsilyloxy)-2-oxopropyl  
8-methyl-3-oxononanoate (4.33)**



**METHOD A: USING  $\text{SiO}_2$**

The crude diketoester (4.33) (250 mg [*ca* 66% pure], *ca* 0.44 mmol) as prepared above, was subjected to radial chromatography as described previously for the cyclisation of the  $\beta$ -ketoester (4.6). The 2-(6-methylheptanoyl)-3-(*t*-butyldimethylsilyloxy)methylbut-2-enolide (4.34) appeared as a pale yellow oil [126 mg, *ca* 65% based on 8-methyl-3-oxononanoic acid (4.30)]. A sample for analysis was further purified, with *ca* 40% loss, by radial chromatography (1 mm  $\text{SiO}_2$ , 15% EtOAc in hexane).

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.08 (s, 6H,  $\text{SiCH}_3$ ), 0.84 (d,  $J = 6.6$  Hz, 6H, 2 x  $\text{CH}_3$ ), 0.89 (s, 9H, *t*-Bu), 1.10–1.23 (m, 2H, H10), 1.23–1.40 (m, 2H, H9), 1.45–1.65 (m, 3H, H8,11), 2.96 (t,  $J = 7.3$  Hz, 2H, H7), 4.98 (s, 2H, H5), 5.05 (s, 2H, H4).

$^{13}\text{C}$  NMR (50.3 MHz,  $\text{CDCl}_3$ )  $\delta$  -5.7 ( $\text{SiCH}_3$ ), 18.1 (*t*-Bu quat.), 22.5 (2 x  $\text{CH}_3$ ), 23.3 (C8), 25.6 (*t*-Bu), 26.8 (C9), 27.8 (C11), 38.7 (C10), 41.6 (C7), 61.8 (C5), 70.2 (C4), 122.4 (C2), 170.7 (C3), 181.2 (C1), 197.1 (C6).

IR  $\nu_{\text{max}}$  ( $\text{CCl}_4$ ) 2960 s, 2940 s, 1775 s (lactone C=O), 1690 m (ketone C=O), 1625 m, 1470 m, 1370 m, 1260 m, 1100 br, 1010 m  $\text{cm}^{-1}$ .

UV  $\lambda_{\text{max}}$  (MeOH) 236 nm ( $\epsilon$  8000), 314 nm ( $\epsilon$  3300); (0.005 M NaOH in MeOH) 220 nm (sh,  $\epsilon$  *ca* 9000), 314 nm ( $\epsilon$  10000).

EIMS  $m/z$  354 ( $\text{M}^+$ , 7%), 339 ( $\text{M}^+ - \text{CH}_2$ , 2), 297 ( $\text{M}^+ - \text{C}_4\text{H}_9$ , 16), 228 (6), 225 (3), 213 (9), 199 (16), 183 (11), 171 (12), 169 (14), 153 (10), 109 (22), 105 (11), 83 (14), 75 (100), 73 (73), 69 (11), 57 (36), 55 (19), 43 (43).

Analysis: Found C 64.4, H 9.4;  $\text{C}_{19}\text{H}_{34}\text{O}_4\text{Si}$  requires C 64.4, H 9.7.

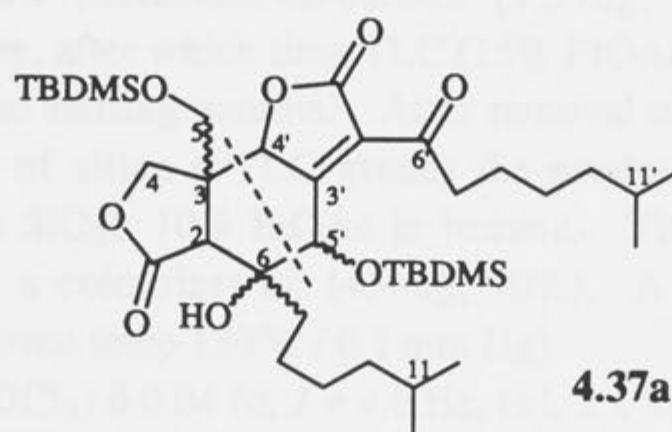


## METHOD B: USING NaH

The crude diketoester (**4.33**) (500 mg [*ca* 66% pure], *ca* 0.88 mmol) as prepared above was cyclised with sodium hydride in THF as previously described for the cyclisation of the diketoester **4.6**.

Chromatography of the product mixture gave the butenolide (**4.34**) (234 mg, 60% based on 8-methyl-3-oxononanoic acid **4.30**) as the first eluted product. (See above for spectroscopic data).

A second minor band contained the tricyclic dimer (**4.37a**) (20 mg, 5% based on **4.30**) which formed colourless prisms (mp 130-132°C) after recrystallisation from pentane.



$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  -0.02, 0.08, 0.09, 0.12 (4 x s, 12H, 4 x  $\text{SiCH}_3$ ), 0.80-0.93 (m, 30H, 2 x *t*-Bu + 4 x  $\text{CH}_3$ ), 1.12-1.42 (m, 10H, H8,9,10,9',10'), 1.43-1.67 (m, 4H, H11,8',11'), 1.86 (m, 1H, H7b), 2.25 (m, 1H, H7a), 2.67 (s, 1H, H2), 2.96 (m, 2H, H7'), 3.62 (d,  $J_{5a,5b} = 10.0$  Hz, 1H, H5b), 3.79 (d,  $J_{5a,5b} = 10.0$  Hz, 1H, H5a), 3.79 (d,  $J_{4a,4b} = 9.0$  Hz, 1H, H4b), 3.86 (d,  $J_{4a,4b} = 9.0$  Hz, 1H, H4a), 5.34 (s, 1H, H5')\*, 5.38 (s, 2H, H4')\*.

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  -5.46, -5.46, -5.37, -4.69 (4 x  $\text{SiCH}_3$ ), 17.8, 18.6 (2 x *t*-Bu quat.), 21.9 (C8), 22.5 (4 x  $\text{CH}_3$ ), 23.5 (C8'), 25.6, 25.9 (2 x *t*-Bu), 26.8 (C9)\*, 27.2 (C9')\*, 27.8 (C11, C11'), 34.4 (C7), 38.6 (C10), 39.0 (C10'), 42.1 (C7'), 49.4 (C2), 52.1 (C3), 67.3 (C5)\*, 67.4 (C5'), 68.4 (C4)\*, 76.9 (C4'), 80.6 (C6), 126.6 (C2'), 169.2 (C3'), 174.9 (C1)\*, 176.3 (C1')\*, 197.7 (C6').

$^1\text{H}$  and  $^{13}\text{C}$  assignments made using  $^1\text{H}$ - $^1\text{H}$  (COSY) and  $^1\text{H}$ - $^{13}\text{C}$  correlated (HETCOR) 2D spectra [see Appendices Ai) and Aii)].

UV  $\lambda_{\text{max}}$  (MeOH) 236 nm ( $\epsilon$  770), 317 nm ( $\epsilon$  180); (0.005 M NaOH in MeOH) 245 nm ( $\epsilon$  6000), 317 nm (7800).

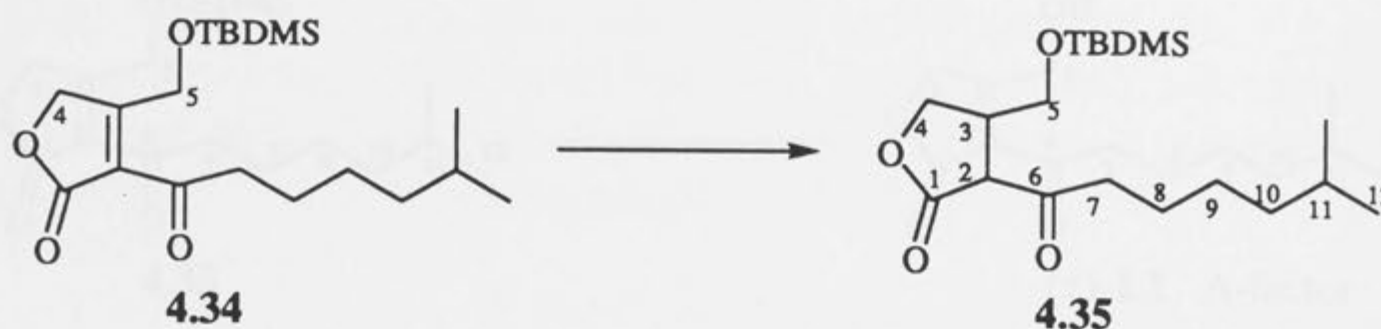
EIMS  $m/z$  651 ( $\text{M}^+ - \text{C}_4\text{H}_9$ , 89%), 633 (19), 621 (3), 607 (8), 519 (2), 353 (s), 127 (3), 109 (17), 89 (23), 75 (57), 73 (100), 57 (50), 55 (22), 43 (53).

CIMS  $m/z$  726 ( $\text{M} + \text{NH}_4^+$ , 9%), 709 ( $\text{M} + \text{H}^+$ , 12).

Analysis: Found C 64.3, H 10.0;  $\text{C}_{38}\text{H}_{68}\text{O}_8\text{Si}_2$  requires C 64.4, H 9.7.



**3-(*t*-Butyldimethylsilyloxymethyl)-2-(6-methylheptanoyl)butanolide (4.35)**  
*[i.e.* TBDMS protected A-Factor]



The butenolide (**4.34**) (50 mg, 0.141 mmol) in ethyl acetate (4 mL) was hydrogenated over 10% palladium-on-carbon (1.5 mg) for 3 hours at ambient temperature and pressure, after which time TLC (15% EtOAc in hexane; **4.34**  $R_f$  0.55; **4.35**  $R_f$  0.43) showed no starting material. After removal of the catalyst by filtration through a 0.4 cm bed of silica (PTLC grade) the product was purified by radial chromatography (1 mm SiO<sub>2</sub>; 10% EtOAc in hexane). The silyl-protected A-factor (**4.35**) was isolated as a colourless oil (45 mg, 90%). A sample for analysis was distilled, bulb-to-bulb (oven temp 130°C / 0.1 mm Hg).

<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  0.04 (d,  $J$  = 4.0 Hz, 6H, 2 x SiCH<sub>3</sub>), 0.85 (d,  $J$  = 6.8 Hz, 6H, 2 x CH<sub>3</sub>), 0.87 (s, 9H, *t*-Bu), 1.14–1.20 (m, 2H, H10), 1.25–1.33 (m, 2H, H9), 1.47–1.55 (m, 1H, H11), 1.55–1.60 (m, 2H, H8), 2.61 (dt,  $J_{7a,7b}$  = 17.6 Hz,  $J_{7b,8}$  = 7.2 Hz, 1H, H7b), 2.94 (dt,  $J_{7a,7b}$  = 17.6 Hz,  $J_{7a,8}$  = 7.2 Hz, 1H, H7a), 3.17 (m, 1H, H3), 3.62 (‘d’,  $J$  = 4.4 Hz, 2H, H5), 3.64 (d,  $J_{2,3}$  = 6.8 Hz, 1H, H2), 4.12 (dd,  $J_{4a,4b}$  = 8.8 Hz,  $J_{4b,3}$  = 5.8 Hz, 1H, H4b), 4.39 (dd,  $J_{4a,3}$  = 7.8 Hz,  $J_{4a,4b}$  = 8.8 Hz, 1H, H4a), 11.2 (s, enol OH), [ca 5% enol].

<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  -6.0 (2 x SiCH<sub>3</sub>), 17.8 (*t*-Bu quat.), 22.3 (2 x CH<sub>3</sub>), 23.2 (C8), 25.4 (*t*-Bu), 26.5 (C9), 27.5 (C11), 38.4 (C10), 39.1 (C3), 42.3 (C7), 54.5 (C2), 61.8 (C5), 69.1 (C4), 172.8 (C1), 203.2 (C6). A minor set of peaks were assigned as the enol tautomer **4.36** [content ca 5%] <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  32.6 (C7), 39.3 (C3), 64.8 (C5), 70.0 (C4), 96.0 (C2), 173.2 (C1)\*, 176.8 (C6)\* (remaining peaks obscured). <sup>1</sup>H and <sup>13</sup>C peak assignments for the enol tautomer **4.36** were confirmed using 2D <sup>1</sup>H-<sup>1</sup>H (COSY) and <sup>1</sup>H-<sup>13</sup>C (HETCOR) spectra. [See Appendices Bi) and ii)].

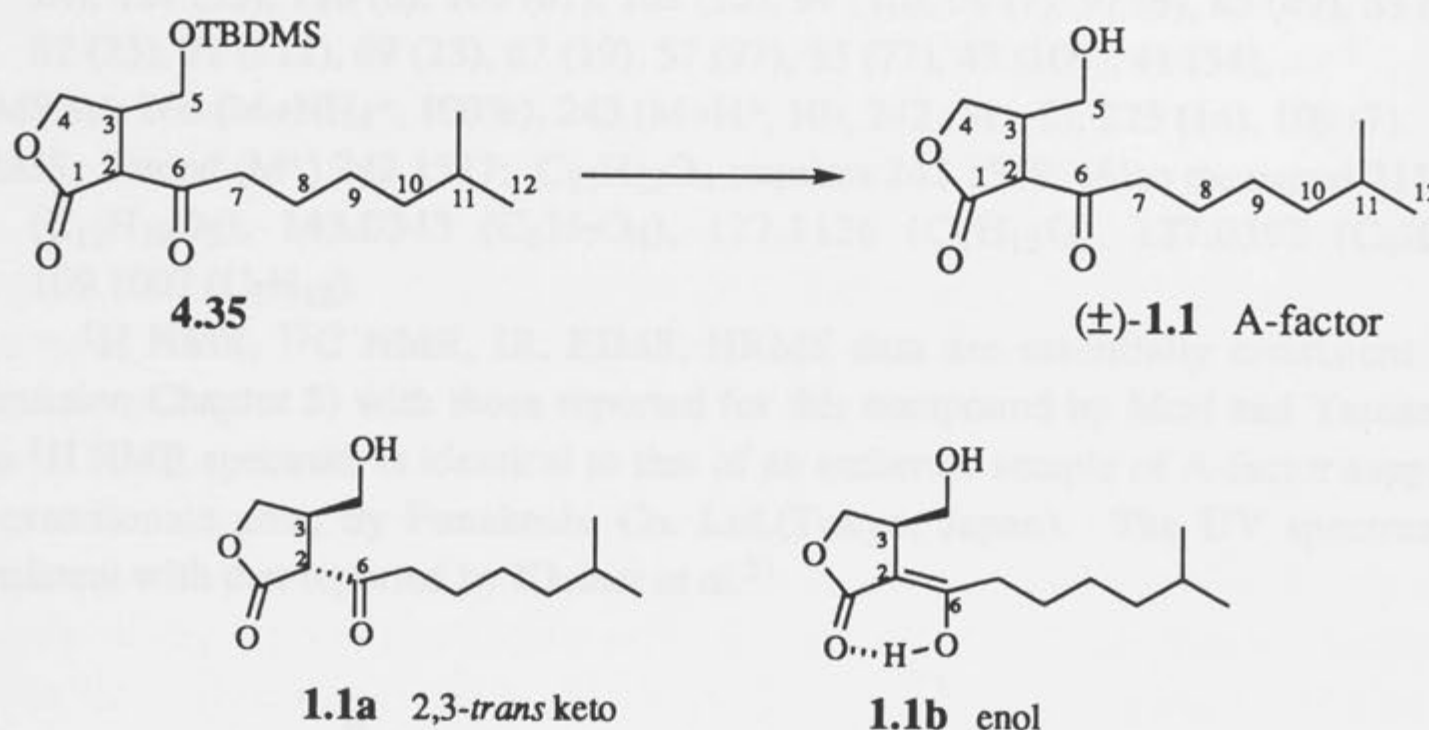
FTIR (CCl<sub>4</sub>)  $\nu_{\max}$  2957 s, 2931 s, 2859 m, 1779 s (lactone C=O), 1720 s (ketone C=O), 1648 w, 1470 m, 1387 m, 1365 m, 1256 m, 1218 m, 1167 m, 1109 m, 1034 m, 1004 m, 939 w cm<sup>-1</sup>.

UV  $\lambda_{\max}$  (MeOH) 256 nm ( $\epsilon$  1100); 0.005 M NaOH in MeOH) 283 nm ( $\epsilon$  20000).

EIMS  $m/z$  341 (M<sup>+</sup>-CH<sub>3</sub>, 1%), 301 (1), 300 (3.8), 299 (M<sup>+</sup>-C<sub>4</sub>H<sub>9</sub>, 19), 281 (3), 225 (2), 201 (12), 173 (4), 161 (4), 157 (5), 149 (8), 127 (30), 109 (68), 105 (11), 89 (10), 83 (12), 75 (60), 73 (41), 57 (53), 55 (32), 43 (100).

Analysis: Found C 64.1, H 10.5; C<sub>19</sub>H<sub>36</sub>O<sub>4</sub>Si requires C 64.0, H 10.2.



3-Hydroxymethyl-2-(6-methylheptanoyl)butanolide [*i.e.* 'A-factor' (1.1)]**METHOD A: DESILYLATION WITH GLACIAL ACETIC ACID : THF : H<sub>2</sub>O (3 : 3 : 1)**

The TBDMS protected butanolide (**4.35**) (100 mg, 0.28 mmol) was desilylated as described above the 2-heptanoyl butanolide (**3.36**, Method A). After chromatography, 15 mg (15%) of unreacted silyl butanolide (**4.35**) was recovered, followed by A-factor (**1.1**) (41 mg, 61%) as a colourless oil, a sample of which was immediately analysed by NMR spectroscopy.

**Data for 1.1a / 1.1b:**

<sup>1</sup>H NMR (500 MHz, acid-free CDCl<sub>3</sub>) δ 0.86 (d, *J* = 6.6 Hz, 6H, 2 x CH<sub>3</sub>), 1.13-1.24 (m, 2H, H<sub>10</sub>), 1.24-1.35 (m, 2H, H<sub>9</sub>), 1.46-1.55 (m, 1H, H<sub>11</sub>), 1.55-1.64 (m, 2H, H<sub>8</sub>), 2.64 (dt, *J*<sub>7a,7b</sub> = 17.6 Hz, *J*<sub>7b,8</sub> = 7.3 Hz, 1H, H<sub>7b</sub>), 2.98 (dt, *J*<sub>7a,7b</sub> = 17.6 Hz, *J*<sub>7a,8</sub> = 7.5 Hz, 1H, H<sub>7a</sub>), 3.25 (m, 1H, H<sub>3</sub>), 3.67 (d, *J*<sub>2,3</sub> = 6.9 Hz, 1H, H<sub>2</sub>), 3.68 (dd, *J*<sub>5b,3</sub> = 5.4 Hz, *J*<sub>5a,5b</sub> 10.7 Hz, 1H, H<sub>5b</sub>), 3.73 (dd, *J*<sub>5a,3</sub> = 5.4 Hz, *J*<sub>5a,5b</sub> 10.7 Hz, 1H, H<sub>5a</sub>), 4.14 (dd, *J*<sub>4a,4b</sub> = 9.0 Hz, *J*<sub>4b,3</sub> = 6.7 Hz, 1H, H<sub>4b</sub>), 4.43 (dd, *J*<sub>4a,4b</sub> = 9.0 Hz, *J*<sub>4a,3</sub> = 8.2 Hz, 1H, H<sub>4a</sub>), 11.27 (s, enol OH), [3% enol tautomer **1.1b**]. Additional complex minor peaks are attributed to *ca* 10% each of the hemiacetal tautomers (**5.12a** and **5.12b**), the spectroscopic data for which appear below.

<sup>13</sup>C NMR (75 MHz, acid-free CDCl<sub>3</sub>) δ 22.3 (2 x CH<sub>3</sub>), 23.3 (C<sub>8</sub>), 26.5 (C<sub>9</sub>), 27.6 (C<sub>11</sub>), 38.5 (C<sub>10</sub>), 39.0 (C<sub>3</sub>), 42.4 (C<sub>7</sub>), 54.8 (C<sub>2</sub>), 61.7 (C<sub>5</sub>), 70.0 (C<sub>4</sub>), 172.8 (C<sub>1</sub>), 203.5 (C<sub>6</sub>). A minor set of peaks were assigned as the enol tautomer **1.1b** [content *ca* 5%] <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 32.9 (C<sub>7</sub>), 64.4 (C<sub>5</sub>), 70.0 (C<sub>4</sub>), 95.5 (C<sub>2</sub>), 173.7 (C<sub>1</sub>)\*, 176.6 (C<sub>6</sub>)\* (remaining peaks obscured).

FTIR (CCl<sub>4</sub>) ν<sub>max</sub>. 3639 m (primary OH), 3606 m (tertiary OH), 3500 w br, 2956 s, 2870 m, 1782 s (lactone C=O), 1720 s (ketone C=O), 1647 w (chelated C=O), 1468 m, 1385 m (*gem*-dimethyl), 1367 m (*gem*-dimethyl), 1217 m, 1177 s, 1031 s, 962 w, 910 w, cm<sup>-1</sup>.



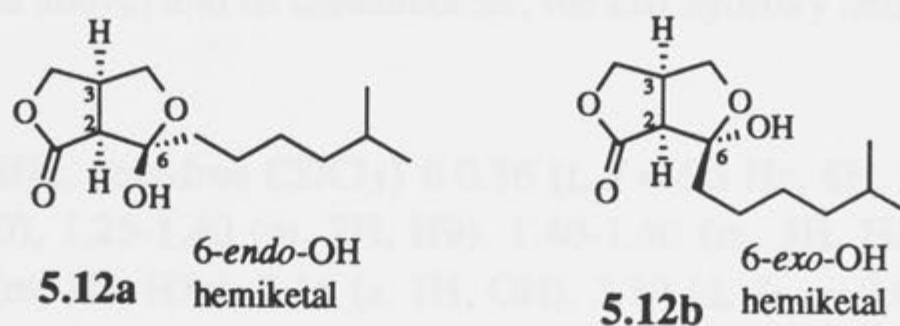
UV  $\lambda_{\text{max}}$  (MeOH) 257 nm ( $\epsilon$  1200); (0.005 M NaOH in MeOH) 283 nm ( $\epsilon$  20100).

EIMS  $m/z$  242 ( $M^+$ , 0.3), 227 ( $M^+ - \text{CH}_3$ , 0.3), 224 ( $M^+ - \text{H}_2\text{O}$ , 0.5) 211 ( $M^+ - \text{CH}_2\text{OH}$ , 4), 193 (0.5), 185 (0.4), 171 ( $M^+ - \text{C}_5\text{H}_{11}$ , 2), 159 (2), 158 (3), 153 (8), 143 ( $M^+ - \text{C}_7\text{H}_{15}$ , 24), 127 (23), 116 (6), 109 (61), 102 (12), 99 (10), 98 (7), 97 (6), 85 (49), 83 (22), 82 (25), 71 (112), 69 (23), 67 (19), 57 (97), 55 (77), 43 (100), 41 (54).

CIMS  $m/z$  260 ( $M + \text{NH}_4^+$ , 100%), 243 ( $M + \text{H}^+$ , 10), 242 ( $M^+$ , 8), 225 (14), 109 (7).

HRMS: Found ( $M^+$ ) 242.1517;  $\text{C}_{13}\text{H}_{22}\text{O}_4$  requires 242.1518; Also measured 211.133 ( $\text{C}_{12}\text{H}_{19}\text{O}_3$ ), 143.0343 ( $\text{C}_6\text{H}_7\text{O}_4$ ), 127.1126 ( $\text{C}_8\text{H}_{15}\text{O}$ ), 127.0392 ( $\text{C}_6\text{H}_7\text{O}_3$ ), 109.1007 ( $\text{C}_8\text{H}_{13}$ ).

$^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, IR, EIMS, HRMS data are essentially consistent (see discussion Chapter 5) with those reported for this compound by Mori and Yamane.<sup>18</sup> The  $^1\text{H}$  NMR spectrum is identical to that of an authentic sample of A-factor supplied, at extortionate cost, by Funakoshi Co. Ltd. (Tokyo, Japan). The UV spectrum is consistent with that reported by Kleiner *et al.*<sup>51</sup>



#### METHOD B: DESILYLATION WITH $\text{HF}_{\text{AQ}}$ / PYRIDINE / $\text{CH}_3\text{CN}$

Using the method described above for the synthesis of 11-demethyl A-factor (3.36) (Method B), the TBDMS protected butanolide (4.35) (50 mg, 0.14 mmol) was desilylated to give A-factor (32 mg, 95%) as a colourless oil.  $^1\text{H}$  and  $^{13}\text{C}$  NMR analyses performed immediately following chromatography gave identical data to that described for A-factor (1.1) above (Method A). Upon standing at  $4^\circ\text{C}$  for several weeks, the product became a waxy solid which showed, by  $^1\text{H}$  NMR, to contain increased levels of the bicyclic hemiacetal tautomer (5.12a). A sample of the product was dissolved in  $\text{CCl}_4$  (ca 20  $\mu\text{L}/\text{mg}$ ) then was cooled slowly to  $-5^\circ\text{C}$  for 24 hours to form the pure, crystalline bicyclic hemiacetal tautomer (5.12a) as colourless plates (mp  $69\text{--}72^\circ\text{C}$ ). NMR spectra of this tautomer alone, were obtained using acid-free  $\text{CDCl}_3$  at  $-20^\circ\text{C}$  which retarded tautomerisation.

$^1\text{H}$  NMR (300 MHz, acid-free  $\text{CDCl}_3$ ,  $-20^\circ\text{C}$ )  $\delta$  0.86 (d,  $J = 6.5$  Hz, 6H, 2 x  $\text{CH}_3$ ), 1.10–1.25 (m, 2H, H10), 1.25–1.38 (m, 2H, H9), 1.38–1.58 (m, 3H, H11,8), 1.86–1.96 (m, 2H, H7), 2.68 (d,  $J_{2,\text{OH}} = 1$  Hz, 1H, OH), 3.13 (dd,  $J_{2,3} = 10.8$  Hz,  $J_{2,\text{OH}} = 1$  Hz, 1H, H2), 3.41 (m, 1H, H3), 4.01 (dd,  $J_{5a,5b} = 9.0$  Hz,  $J_{5b,3} = 5.2$  Hz, 1H, H5b), 4.20 (dd,  $J_{5a,5b} = 9.0$  Hz,  $J_{5a,3} = 8.2$  Hz, 1H, H5a), 4.26 (dd,  $J_{4a,4b} = 9.0$  Hz,  $J_{4b,3} = 6.2$  Hz, 1H, H4b), 4.49 (dd,  $J_{4a,4b} = 9.0$  Hz,  $J_{4a,3} = 9.0$  Hz, 1H, H4a).



$^{13}\text{C}$  NMR (75 MHz, acid-free  $\text{CDCl}_3$ )  $\delta$  22.3 (2 x  $\text{CH}_3$ ), 23.5 (C8), 27.1 (C9), 27.6 (C11), 38.6 (C10)\*, 38.8 (C7)\*, 40.8 (C3), 53.7 (C2), 72.0 (C5)\*, 72.6 (C4)\*, 106.9 (C6), 175.0 (C1) (peak multiplicities determined by APT spectrum).

FTIR (KBr disc)  $\nu_{\text{max}}$  3395 s (OH), 2955 s, 1743 s (lactone C=O), 1468 m, 1397 s, 1366 m, 1228 s, 1211 s, 1155 s, 1116 m, 1031 s, 985 s, 741 m, 705  $\text{cm}^{-1}$ .

EIMS (identical to that reported for **1.1** above).

HRMS: Found ( $\text{M}^+$ ) 242.1517;  $\text{C}_{13}\text{H}_{22}\text{O}_4$  requires 242.1518.

The X-ray crystal structure was determined on crystals obtained from  $\text{CH}_2\text{Cl}_2$  / pentane (40:60, ca 50  $\mu\text{L}/\text{mg}$ ), mp 69–72°C, [see Fig 5.3, and Appendix Ei]. The structure (**5.12a**) confirmed the C6-tertiary hydroxyl group was in the *endo* position. Crystallographic data including a table of selected interatomic distances and angles appear in Appendix Eii.

Another sample of the product was crystallised from pentane- $\text{CH}_2\text{Cl}_2$  (1 : 1) by slow evaporation at -5°C to give an amorphous colourless powder (mp 40–60°C) which was shown by  $^1\text{H}$  NMR to contain a 55 : 45 mixture of the *endo*-hydroxy hemiacetal (**5.12a**) (described above) and its diastereomer, the *exo* hydroxy hemiacetal (**5.12b**).

#### Data for **5.12b**:

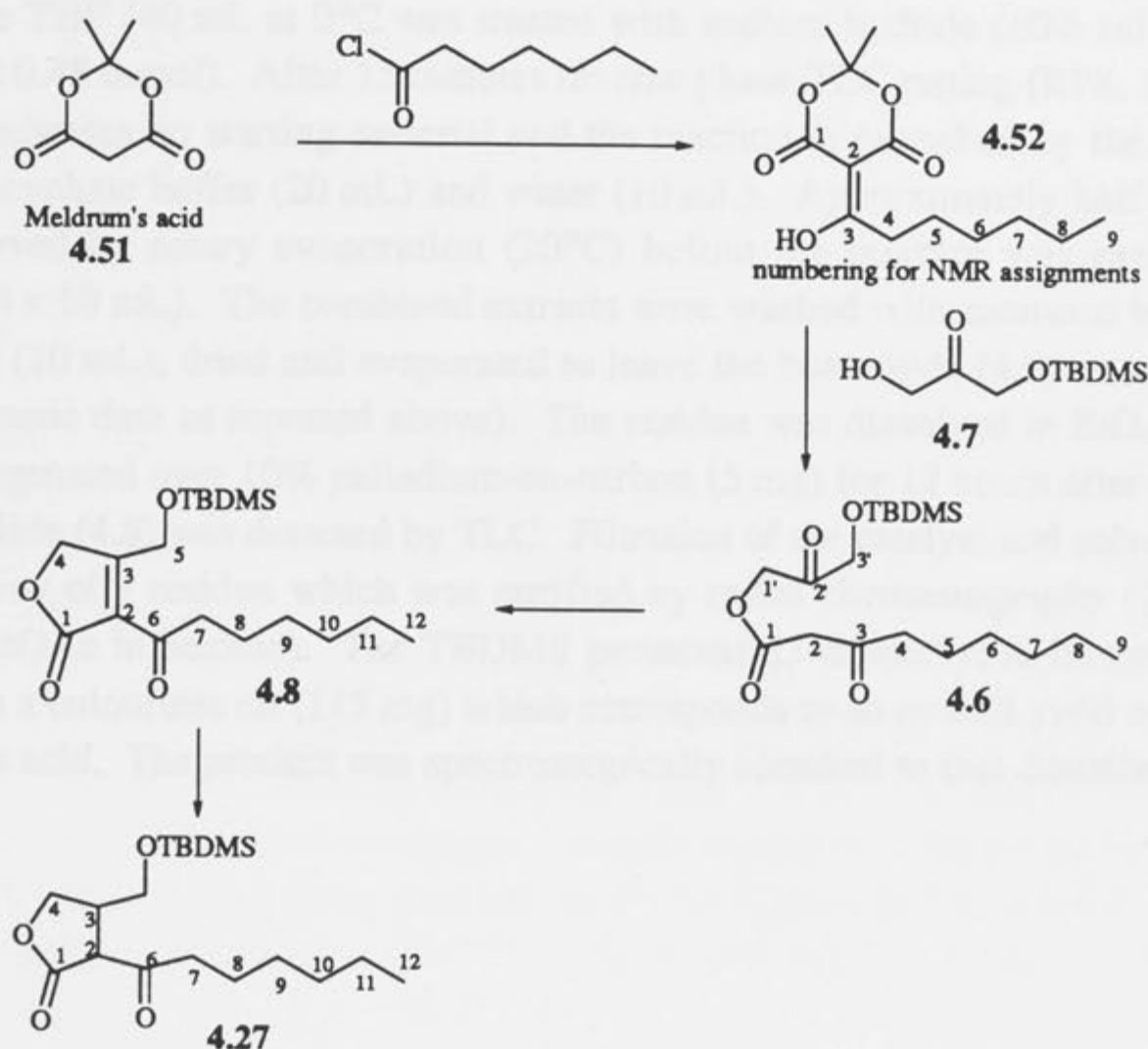
$^1\text{H}$  NMR (300 MHz, acid-free  $\text{CDCl}_3$ )  $\delta$  0.86 (t,  $J = 6.5$  Hz, 6H, 2 x  $\text{CH}_3$ ), 1.10–1.25 (m, 2H, H10), 1.25–1.40 (m, 2H, H9), 1.40–1.60 (m, 3H, H8,11), 1.77 (m, 1H, H7b), 2.05 (m, 1H, H7a), 2.16 (s, 1H, OH), 3.19 (d,  $J_{2,3} = 9.0$  Hz, 1H, H2), 3.41 (m, 1H, H3), 3.87 (dd,  $J_{5a,5b} = 9.2$  Hz,  $J_{5b,3} = 1.7$  Hz, 1H, H5b), 4.10 (dd,  $J_{5a,5b} = 9.2$  Hz,  $J_{5a,3} = 4.8$  Hz, 1H, H5a), 4.16 (dd,  $J_{4a,4b} = 9.2$  Hz,  $J_{4b,3} = 6.2$  Hz, 1H, H4b), 4.48 (dd,  $J_{4a,4b} = 9.2$  Hz,  $J_{4a,3} = 9.0$  Hz, 1H, H4a).

$^{13}\text{C}$  NMR (75 MHz, acid-free  $\text{CDCl}_3$ )  $\delta$  22.3 (2 x  $\text{CH}_3$ ), 23.6 (C8), 27.2 (C9), 27.6 (C11), 36.5 (C7), 38.6 (C10), 39.5 (C3), 53.6 (C2), 71.4 (C5)\*, 72.4 (C4)\*, 107.6 (C6), 175.1 (C1).

After 1 week at room temperature the NMR sample had reached an equilibrium between the four tautomeric forms of A-factor, which was 62. : 17 : 17 : 4 [2,3-*trans* keto (**1.1a**) : *endo*-hydroxy hemiacetal (**5.12a**) : *exo*-hydroxy hemiacetal (**5.12b**) : enol form (**1.1b**)].



## 11-Demethyl A-Factor (3.36) from Meldrum's acid (4.51)



The required  $\beta$ -ketoester (4.6) was prepared following the general method of Oikawa *et al.*<sup>162</sup> Thus, a stirred solution of Meldrum's acid (4.51) (0.648 g, 4.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was cooled to 0°C and treated sequentially with pyridine (0.712 g) and a solution of heptanoyl chloride (0.72 g, 4.8 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL). The resulting mixture became orange and was stirred for 1 hour at 0°C then at room temperature for a further 1 hour. Dilute aqueous hydrochloric acid (1 M, 10 mL) was added and the phases were separated. The aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and the combined organic phase was washed well with water (4 x 5 mL). Drying and removal of the solvent left the crude heptanoyl derivative (4.52) as an orange oil (1.13 g).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.86 (t,  $J$  = 6.6 Hz, 3H, H<sub>9</sub>), 1.20-1.47 (m, 6H, H<sub>6,7,8</sub>), 1.55-1.78 (m, 2H, H<sub>5</sub>), 1.70 (s, 6H, C(CH<sub>3</sub>)<sub>2</sub>), 3.04 (t,  $J$  = 7.6 Hz, 2H, H<sub>4</sub>), 15.2 (s, 1H, enolic OH), [100% enol].

The crude acyl Meldrum's acid (1.13 g) in benzene (7 mL) was combined with TBDMS protected dihydroxyacetone (4.7) (2.4 g, 11.8 mmol) which had been converted to its monomer by brief heating to *ca* 150°C (5 min). The mixture was heated at reflux for 2 hours and then the solvent was removed by rotary evaporation. Unreacted silyl dihydroxyacetone (4.7) and heptanoic acid were removed by Kugelrohr distillation (oven temp 100°C / 0.1 mm Hg) to leave the crude  $\beta$ -ketoester (4.6) (1.30 g) as a yellow-orange oil. <sup>1</sup>H NMR analysis using an internal integration standard (diphenylmethanol) indicates a purity of *ca* 85% and thus a yield of *ca* 69% from Meldrum's acid. <sup>1</sup>H and <sup>13</sup>C NMR data are identical to that reported above for the

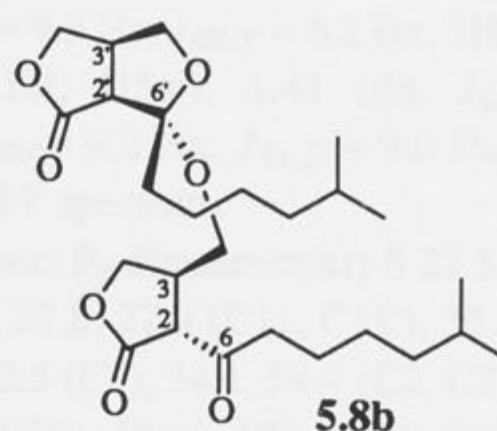
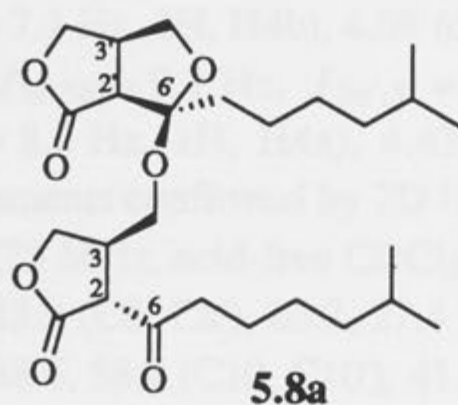


$\beta$ -ketoester (4.6). The product is stable on storage at 4°C and is suitable for cyclisation without further purification.

The crude  $\beta$ -ketoester (4.6) (200 mg, *ca* 0.47 mmol) prepared above in anhydrous THF (40 mL at 0°C was treated with sodium hydride (60% oil dispersion, 19 mg, *ca* 0.48 mmol). After 15 minutes reverse phase TLC testing (RP8, 10% H<sub>2</sub>O in MeOH) indicates no starting material and the reaction is quenched by the addition of pH 5.0 phosphate buffer (20 mL) and water (10 mL). Approximately half of the THF was removed by rotary evaporation (20°C) before the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 10 mL). The combined extracts were washed with saturated brine (5 mL) and water (10 mL), dried and evaporated to leave the butenolide (4.8) as an orange oil (spectroscopic data as reported above). The residue was dissolved in EtOAc (15 mL) and hydrogenated over 10% palladium-on-carbon (5 mg) for 12 hours after which time no butenolide (4.8) was detected by TLC. Filtration of the catalyst and solvent removal left a yellow oily residue which was purified by radial chromatography (2 mm SiO<sub>2</sub>; 10-20% EtOAc in hexane). The TBDMS protected 11-demethyl A-factor (4.27) was isolated as a colourless oil (115 mg) which corresponds to an overall yield of 48% from Meldrum's acid. The product was spectroscopically identical to that described above.



## Dimerisation of A-factor (1.1)



A slow stream of HCl gas was briefly (*ca* 3 sec) bubbled through a solution of A-factor (1.1) (25 mg) in acid-free  $\text{CDCl}_3$  (0.3 mL). After 1 hour, TLC analysis (1% MeOH in  $\text{CH}_2\text{Cl}_2$ ) showed the appearance of 4 new less polar compounds [ $R_f$  values 0.53, 0.50, 0.44, 0.38; A-factor (1.1)  $R_f$  0.15]. Removal of the solvent followed by careful radial chromatography (1 mm  $\text{SiO}_2$ ) using a mildly basic eluent system (0-1% MeOH and 0.1% pyridine in  $\text{CH}_2\text{Cl}_2$ ), separated the new compounds into 3 fractions, followed by a band containing an unchanged A-factor (1.1) (7 mg).

The first eluted band contained 2 diastereomers of **5.8b** (7 mg) in which the 2,3-*trans* A-factor portion is attached *exo*- to the fused bicyclic ring system:

$^1\text{H}$  NMR (300 MHz, acid-free  $\text{CDCl}_3$ ) (2 diastereomers)  $\delta$  0.85 (d,  $J = 6.6$  Hz, 24H, 8 x  $\text{CH}_3$ ), 1.1-2.0 (m, 2 x 16H, 2 x  $\text{H}8',9',10',11'+2$  x  $\text{H}7,8,9,10,11$ ), 2.53-2.67 (m, 2 x 1H, 2 x  $\text{H}7b$ ), 2.87-3.03 (m, 2 x 1H, 2 x  $\text{H}7a$ ), 3.12 (d,  $J_{2,3} = 9.0$  Hz, 1H,  $\text{H}2'$ ), 3.16 (d,  $J_{2,3} = 9.1$  Hz, 1H,  $\text{H}2'$ ), 3.2-3.6 (m, 10H, 2 x  $\text{H}2,3,5,3'$ ), 3.8-4.5 (m, 12H, 2 x  $\text{H}4,4',5'$ ).

The second and third bands (4 mg each) each contained one diastereomer of the alternate type (**5.8a**) in which the 2,3-*trans* portions join *endo*- to the fused bicyclic systems.

**5.8a** (1 diastereomer, higher  $R_f$ ):

$^1\text{H}$  NMR  $\delta$  0.87 (d,  $J = 6.6$  Hz, 12H, 4 x  $\text{CH}_3$ ), 1.10-1.65 (m, 14H), 1.71 (m, 1H,  $\text{H}7b'$ ), 1.96 (m, 1H,  $\text{H}7a'$ ), 2.64 (dt,  $J_{7a,7b} = 18.3$  Hz,  $J_{7b,8} = 7.2$  Hz, 1H,  $\text{H}7b$ ), 2.97 (dt,  $J_{7a,7b} = 18.3$  Hz,  $J_{7a,8} = 7.2$  Hz, 1H,  $\text{H}7a$ ), 3.18 (d,  $J_{2',3'} = 11.0$  Hz, 1H,  $\text{H}2'$ ), 3.22-3.40 (m, 1H,  $\text{H}3$ ), 3.38 (dd,  $J_{5a,5b} = 8.9$  Hz,  $J_{5b,3} = 7.8$  Hz, 1H,  $\text{H}5b$ ), 3.35-3.50 (m, 1H,  $\text{H}3'$ ), 3.56 (d,  $J_{2,3} = 6.7$  Hz, 1H,  $\text{H}2$ ), 3.63 (dd,  $J_{5a,5b} = 8.9$  Hz,  $J_{5a,3} = 5.3$  Hz, 1H,  $\text{H}5a$ ), 3.78 (dd,  $J_{5a',5b'} = 9.1$  Hz,  $J_{5b',3'} = 5.3$  Hz, 1H,  $\text{H}5b'$ ), 3.99 (dd,  $J_{4a,4b} = 8.9$  Hz,  $J_{4b,3} = 8.6$  Hz, 1H,  $\text{H}4b$ ), 4.00 (dd,  $J_{4a',4b'} = 9.2$  Hz,  $J_{4b',3'} = 6.2$  Hz, 1H,  $\text{H}4b'$ ), 4.22 (dd,  $J_{5a',5b'} = 9.1$  Hz,  $J_{5a',3'} = 8.6$  Hz, 1H,  $\text{H}5a'$ ), 4.40 (dd,  $J_{4a,4b} = 8.9$  Hz,  $J_{4a,3} = 7.7$  Hz, 1H,  $\text{H}4a$ ), 4.43 (dd,  $J_{4a',4b'} = 9.2$  Hz,  $J_{4a',3'} = 9.2$  Hz, 1H,  $\text{H}4a'$ ).

**5.8a** (1 diastereomer, lower  $R_f$ ):

$^1\text{H}$  NMR  $\delta$  0.86 (d,  $J = 6.6$  Hz, 12H, 4 x  $\text{CH}_3$ ), 1.10-1.60 (m, 14H), 1.69 (m, 1H,  $\text{H}7b'$ ), 1.96 (m, 1H,  $\text{H}7a'$ ), 2.61 (dt,  $J_{7a,7b} = 18.1$  Hz,  $J_{7b,8} = 7.2$  Hz, 1H,  $\text{H}7b$ ), 2.99 (dt,  $J_{7a,7b} = 18.1$  Hz,  $J_{7a,8} = 7.5$  Hz, 1H,  $\text{H}7a$ ), 3.21 (d,  $J_{2',3'} = 11.1$  Hz, 1H,  $\text{H}2'$ ), 3.22 (m, 1H,  $\text{H}3$ ), 3.43 (m, 1H,  $\text{H}3'$ ), 3.44 (dd,  $J_{5a,5b} = 9.5$  Hz,  $J_{5b,3} = 5.8$  Hz, 1H,  $\text{H}5b$ ),



3.56 (dd,  $J_{5a,5b} = 9.5$  Hz,  $J_{5a,3} = 4.2$  Hz, 1H, H5a), 3.59 (d,  $J_{2,3} = 7.6$  Hz, 1H, H2), 3.79 (dd,  $J_{5a',5b'} = 9.1$  Hz,  $J_{5b',3'} = 5.4$  Hz, 1H, H5b'), 4.04 (dd,  $J_{4a,4b} = 8.9$  Hz,  $J_{4b,3} = 7.1$  Hz, 1H, H4b), 4.08 (dd,  $J_{4a',4b'} = 9.2$  Hz,  $J_{4b',3'} = 6.2$  Hz, 1H, H4b'), 4.25 (dd,  $J_{5a',5b'} = 9.1$  Hz,  $J_{5a',3'} = 8.4$  Hz, 1H, H5a'), 4.41 (dd,  $J_{4a,4b} = 8.9$  Hz,  $J_{4a,3} = 8.2$  Hz, 1H, H4a), 4.43 (dd,  $J_{4a',4b'} = 9.2$  Hz,  $J_{4a',3'} = 9.0$  Hz, 1H, H4a'). Assignments confirmed by 2D  $^1\text{H}$ - $^1\text{H}$  COSY spectrum.

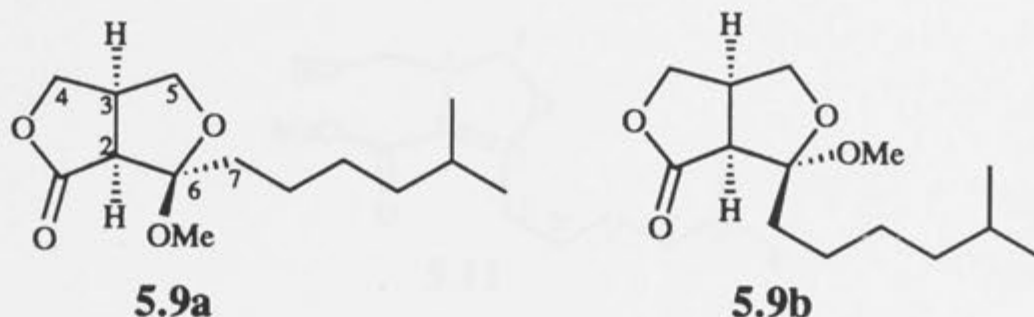
$^{13}\text{C}$  NMR (75 MHz, acid-free  $\text{CDCl}_3$ ) **5.8a** (lower  $R_f$  diastereomer)  $\delta$  22.5 (C12, C12'), 23.4, 23.9 (C8, C8'), 26.7, 27.4 (C9, C9'), 27.8, 27.9 (C11, C11'), 33.7 (C7'), 37.2 (C3), 38.6, 38.7 (C10, C10'), 41.0 (C3'), 42.5 (C7), 54.1, 54.4 (C2, C2'), 59.3 (C5), 69.3 (C4), 72.0 (C5')\*, 72.8 (C4')\*, 109.7 (C6'), 171.8, 173.1 (C1, C1'), 202.7 (C6). Multiplicities determined by APT spectrum, see Appendix C.

EIMS (all 4 diastereomers show identical fragmentations):  $m/z$  367 ( $\text{M}^+ - 99$ , 0.5%), 243 (0.5), 225 (100), 153 (26), 141 (11), 140 (16), 127 (30), 109 (53), 95 (11), 85 (17), 83 (22), 81 (23), 69 (19), 67 (17), 57 (41), 55 (52), 53 (12), 43 (87), 41 (37).

CIMS (all 4 diastereomers show identical fragmentation):  $m/z$  484 ( $\text{M} + \text{NH}_4^+$ , 42%), 467 ( $\text{M} + \text{H}^+$ , 1), 260 (5), 243 (4), 242 (5), 225 (100), 109 (7).



(3 $\alpha$ ,6 $\alpha$ )-6-Methoxy-6-(5-methylhexyl)-1*H*,3*H*-furo[3,4-*c*] tetrahydrofuran-1-one  
(5.9)



To A-factor (**1.1**) (10 mg, 0.041 mmol) in acid-free CDCl<sub>3</sub> (0.8 mL) in an NMR tube [initial tautomer ratio **1.1a** (2,3-*trans*) : **5.12a** (*endo*-OH) : **5.12b** (*exo*-OH), 28 : 44 : 28] was added methanolic HCl (*ca* 0.25 M, 8.5  $\mu$ L). The reaction was followed by <sup>1</sup>H NMR. After 1 hour at 20°C the reaction was quenched by the addition of triethylamine (2  $\mu$ L) and the solvent was evaporated. Radial chromatography of the residual oil (1 mm SiO<sub>2</sub>; 40-50% EtOAc / hexane) yielded the bicyclic methylacetal (**5.9**) (5.1 mg, 50%) as an inseparable 85 : 15 mixture of diastereomers (tentatively assigned *exo*-OMe **5.9b** : *endo*-OMe **5.9a** respectively by comparison of their *J*<sub>2,3</sub> values with those of the hemiacetal forms of A-factor). A final band contained unreacted A-factor (**1.1**) (3 mg, 30%), (spectroscopic data above).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) **5.9b** (major):  $\delta$  0.87 (d, *J* = 6.6 Hz, 6H, 2 x CH<sub>3</sub>), 1.15-1.60 (m, 7H), 1.65 (m, 1H, H7b), 1.98 (m, 1H, H7a), 3.16 (d, *J*<sub>2,3</sub> = 9.1 Hz, 1H, H2), 3.16 (s, 3H, OMe), 3.33 (m, 1H, H3), 3.84 (dd, *J*<sub>5a,5b</sub> = 9.1 Hz, *J*<sub>5b,3</sub> = 2.2 Hz, 1H, H5b), 4.04 (dd, *J*<sub>5a,5b</sub> = 9.1 Hz, *J*<sub>5a,3</sub> = 6.7 Hz, 1H, H5a), 4.13 (dd, *J*<sub>4a,4b</sub> = 9.4 Hz, *J*<sub>4b,3</sub> = 3.9 Hz, 1H, H4b), 4.45 (dd, *J*<sub>4a,4b</sub> = 9.4 Hz, *J*<sub>4a,3</sub> = 8.0 Hz, 1H, H4a).

**5.9a** (minor):  $\delta$  0.87 (d, *J* = 6.6 Hz, 6H, 2 x CH<sub>3</sub>), 1.15-1.62 (m, 7H), 1.73 (m, 1H, H7b), 1.99 (m, 1H, H7a), 3.16 (d, *J*<sub>2,3</sub> = 10.9 Hz, 1H, H2), 3.22 (s, 3H, OMe), 3.43 (m, 1H, H3), 3.80 (dd, *J*<sub>5a,5b</sub> = 8.9 Hz, *J*<sub>5b,3</sub> = 5.2 Hz, 1H, H5b), 4.14 (dd, *J*<sub>4a,4b</sub> = 9.0 Hz, *J*<sub>4b,3</sub> = 6.6 Hz, 1H, H4b), 4.20 (dd, *J*<sub>5a,5b</sub> = 8.9 Hz, *J*<sub>5a,3</sub> = 6.7 Hz, 1H, H5a), 4.45 (dd, *J*<sub>4a,4b</sub> = 9.0 Hz, *J*<sub>4a,3</sub> = 8.0 Hz, 1H, H4a).

<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) **5.9b**:  $\delta$  22.7 (2 x CH<sub>3</sub>), 24.2 (C8), 27.5 (C9), 27.9 (C11), 30.1 (C7), 38.7 (C10), 39.4 (C3), 47.7 (OMe), 53.1 (C2), 71.8 (C5)\*, 72.6 (C4)\*, 110.8 (C6), 174.4 (C1).

**5.9a**:  $\delta$  22.6 (2 x CH<sub>3</sub>), 23.8 (C8), 27.4 (C9), 27.9 (C11), 32.9 (C7), 38.8 (C10), 41.5 (C3), 48.0 (OMe), 54.6 (C2), 72.0 (C5)\*, 72.5 (C4)\*, 109.6 (C6), 174.4 (C1).

Peak multiplicities determined by APT spectrum, see Appendix D.

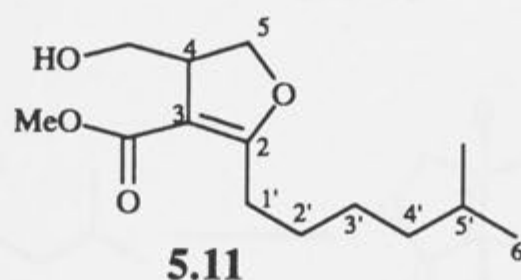
EIMS *m/z* 225 (M<sup>+</sup>-OCH<sub>3</sub>, 16%), 157 (M<sup>+</sup>-C<sub>7</sub>H<sub>15</sub>, 100), 153 (13), 140 (10), 127 (7), 109 (9), 99 (24), 83 (11), 82 (14), 81 (21), 71 (10), 69 (19), 57 (17), 55 (33), 53 (12), 43 (62), 41 (45).

CIMS *m/z* 257 (M+H<sup>+</sup>, 100%).

HRMS: Found (M<sup>+</sup>-OCH<sub>3</sub>) 225.1492; C<sub>13</sub>H<sub>21</sub>O<sub>3</sub> requires 225.1491.



**4-Hydroxymethyl-2-(5-methylhexyl)-4,5-dihydrofuran-3-carboxylic acid methyl ester (5.11)**



A-factor (**1.1**) (4 mg) in anhydrous MeOH (0.5 mL) was treated with methanolic HCl (0.6 M, 0.1 mL) and stirred for 48 hours at room temperature. Triethylamine (10  $\mu$ L) was added to quench the reaction, and after removal of the solvent, the residue was separated by preparative TLC (1% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) into two components. The higher *R<sub>f</sub>* product (*R<sub>f</sub>* 0.43) was identified as a 55 : 45 diastereomeric mixture of the bicyclic methylketals (**5.9a** : **5.9b**, 1.5 mg, 35%; spectroscopic data as above).

The lower *R<sub>f</sub>* product (*R<sub>f</sub>* 0.11) was the carbomethoxy-3,4-dihydrofuran (**5.11**) (1.5 mg, 35%):

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.86 (d, *J* = 6.6 Hz, 6H, 2 x CH<sub>3</sub>), 1.12-1.37 (m, 4H, H<sub>8,9</sub>), 1.45-1.60 (m, 3H, H<sub>7,10</sub>), 2.59-2.67 (m, 2H, H<sub>6</sub>), 3.39 (m, 1H, H<sub>3</sub>), 3.62 (dd, *J*<sub>5a,5b</sub> = 10.6 Hz, *J*<sub>5b,3</sub> = 5.0 Hz, 1H, H<sub>5b</sub>), 3.72 (dd, *J*<sub>5a,5b</sub> = 10.6 Hz, *J*<sub>5a,3</sub> = 6.1 Hz, 1H, H<sub>5a</sub>), 3.74 (s, 3H, CO<sub>2</sub>Me), 4.23 (dd, *J*<sub>4a,4b</sub> = 9.5 Hz, *J*<sub>4b,3</sub> = 5.8 Hz, 1H, H<sub>4b</sub>), 4.47 (dd, *J*<sub>4a,4b</sub> = 9.5 Hz, *J*<sub>4a,3</sub> = 10.2 Hz, 1H, H<sub>4a</sub>).

<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  22.6 (2xCH<sub>3</sub>), 27.1, 27.8, 28.3, 38.5, 45.1, 51.2 (C1'), 65.4 (CH<sub>2</sub>OH), 73.5 (C5), 104.0 (C3), 156.0 (C2), 175.3 (ester C=O). EIMS *m/z* 256 (M<sup>+</sup>, 3%), 225 (M<sup>+</sup>-OCH<sub>3</sub>, 92), 209 (2), 189 (2), 185 (2), 181 (2), 169 (2), 153 (6), 157 (0.7), 147 (3), 141 (3), 127 (14), 109 (12), 97 (10), 95 (13), 83 (15), 81 (29), 71 (11), 69 (47), 67 (12), 59 (51), 57 (51), 55 (57), 43 (100).

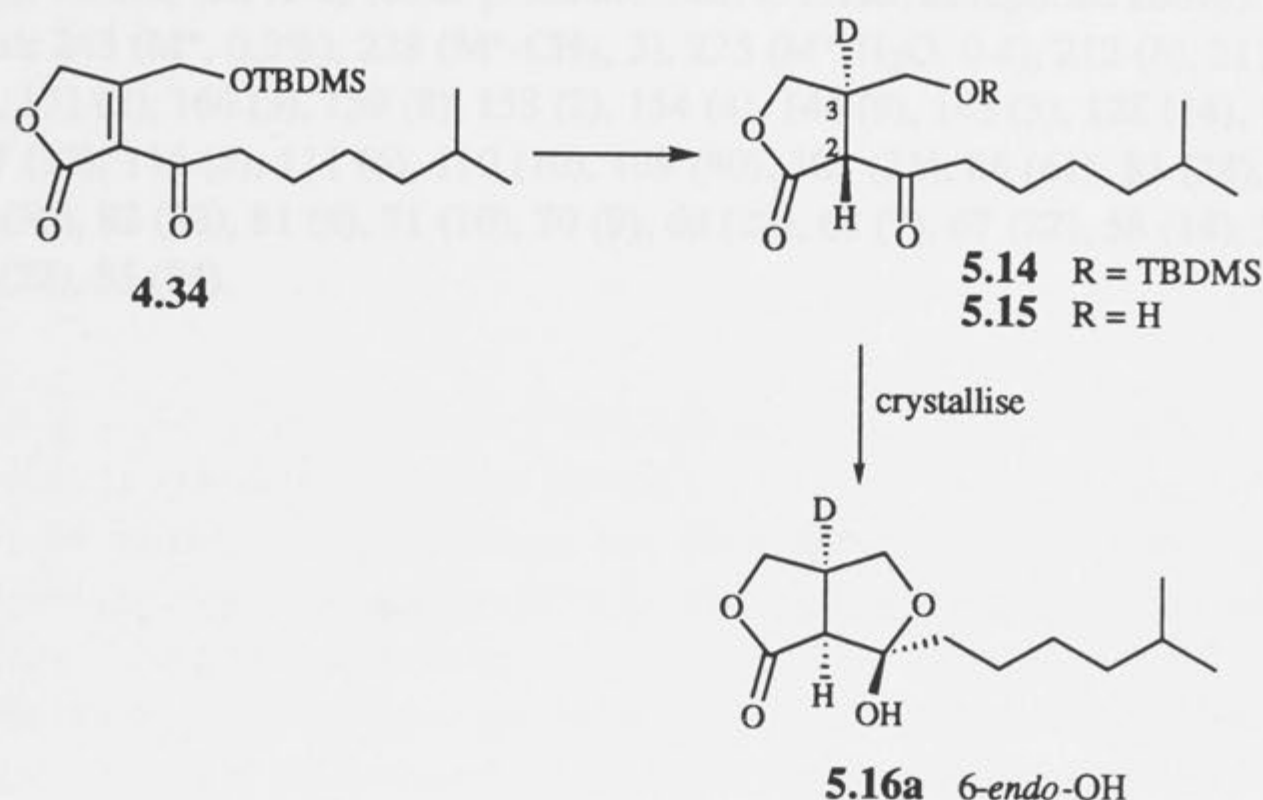
EIMS *m/z* 256 (M<sup>+</sup>, 3%), 226 (15), 225 (92), 153, (6), 127 (14), 109 (12), 97 (10), 95 (13), 83 (15), 81 (29), 71 (11), 69 (47), 67 (12), 59 (52), 57 (51), 55 (57), 53 (15), 43 (100).

CIMS *m/z* 257 (M+H<sup>+</sup>, 100%), 239 (3), 225 (M<sup>+</sup>-CH<sub>2</sub>OH, 55), 153 (4), 123 (4), 109 (9), 99 (7).

HRMS: Found (M<sup>+</sup>) 256.1676; C<sub>14</sub>H<sub>24</sub>O<sub>4</sub> requires 256.1675.



**Preparation of [3-<sup>2</sup>H]-A-factor (5.15) and [3a-<sup>2</sup>H]-(3a $\alpha$ ,6 $\beta$ ,6a $\alpha$ )-6-hydroxy-6-(5-methylhexyl)-1*H*,3*H*-furo[3,4-*c*]tetrahydrofuran-1-one (5.16a)**



The butenolide (**4.34**) (100 mg, 0.282 mmol) in MeOH was stirred with 10% palladium-on-carbon (6 mg) in an atmosphere of deuterium for 45 minutes at ambient temperature and pressure at which time a TLC test showed no starting material. Filtration through PTLC-grade silica gel and removal of solvent gave an oil which was purified by radial chromatography to give [3-<sup>2</sup>H]-TBDMS-protected A-factor (**5.14**) (78 mg, 77%) as a colourless oil.

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>). The product contained *ca* 28% undeuterated silyl protected A-factor (**4.35**) whose data is reported above. Data for the [3-<sup>2</sup>H] compound (**5.14**):-  $\delta$  0.05 (d,  $J$  = 4 Hz, 2 x SiCH<sub>3</sub>), 0.85 (d,  $J$  = 6.8 Hz, 6H, 2 x CH<sub>3</sub>), 0.88 (s, 9H, *t*-Bu), 1.10-1.40 (m, 4H, H<sub>9,10</sub>), 1.45-1.70 (m, 3H, H<sub>8,11</sub>), 2.63 (dt,  $J_{7a,7b}$  = 18.0 Hz,  $J_{7b,8}$  = 7.2 Hz, 1H, H<sub>7b</sub>), 2.96 (dt,  $J_{7a,7b}$  = 18.0 Hz,  $J_{7a,8}$  = 7.2 Hz, 1H, H<sub>7a</sub>), 3.54-3.73 (m, 3H, H<sub>5,2</sub>), 4.13 (d,  $J_{4a,4b}$  = 8.9 Hz, 1H, H<sub>4b</sub>), 4.40 (d,  $J_{4a,4b}$  = 8.9 Hz, 1H, H<sub>4a</sub>).

EIMS  $m/z$  342 (M<sup>+</sup>-CH<sub>3</sub>, 1%), 302 (1.1), 301 (3.5), 300 (M<sup>+</sup>-C<sub>4</sub>H<sub>9</sub>, 14.5), 299 (4.6), 282 (2), 226 (2), 202 (5), 201 (7), 174 (3), 172 (3), 158 (2), 150 (4), 149 (2), 129 (3), 128 (6), 127 (19), 109 (78), 105 (12), 89 (15), 83 (19), 77 (10), 76 (12), 75 (100), 73 (73), 69 (30), 67 (16), 59 (25), 58 (16), 57 (99), 56 (15), 55 (65). Peaks 299, 300, 301, 302 indicate 24% non-deuterated product; 72% mono-deuterated and *ca* 3% di-deuterated, after correction for M + 1 and M + 2 ( $m/z$  300, 301) peaks previously observed for the non-deuterated compound (**4.35**, see above).

The deuterated silyl A-factor (**5.14**) (78 mg) was desilylated with HF / pyridine / CH<sub>3</sub>CN as described for the preparation of 11-demethyl A-factor (**3.36**, Method B), to give [3-<sup>2</sup>H]-A-factor (**5.15**) (42 mg, 80%). Recrystallisation from CCl<sub>4</sub>

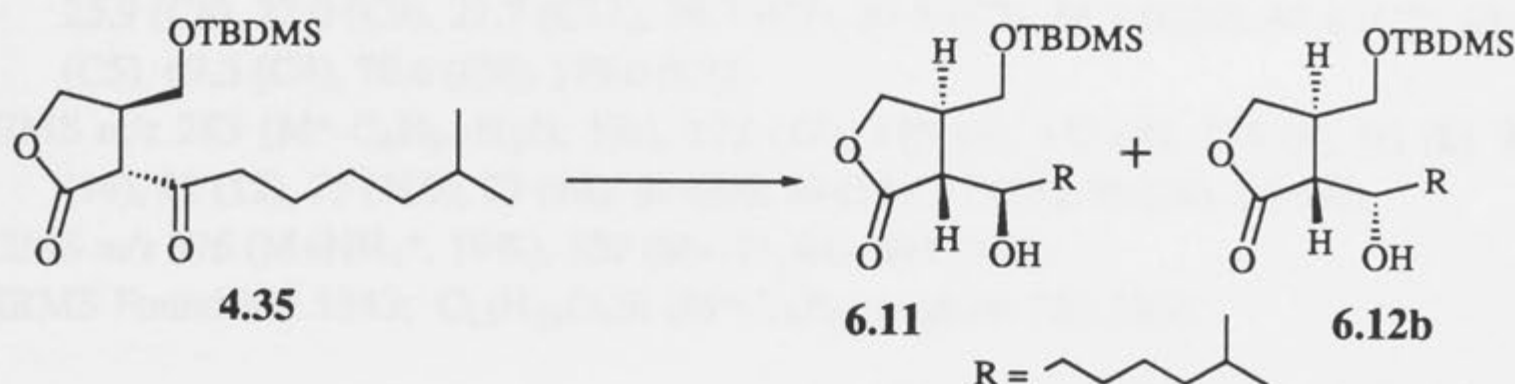


(0.6 mL) at 0°C gave the [3-<sup>2</sup>H]-*endo*-hydroxy hemiacetal tautomer (**5.16a**) as colourless plates (32 mg, mp 65–71°C):

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) Contains *ca* 30% non-deutero A-factor hemiketal (**5.12a**) (data above). Compound **5.16a**: δ 3.13 (s, 1H, H<sub>2</sub>), 4.01 (d, *J*<sub>5a,5b</sub> 9.0 Hz, 1H, H<sub>5b</sub>), 4.20 (d, *J*<sub>5a,5b</sub> 9.0 Hz, 1H, H<sub>5a</sub>), 4.26 (d, *J*<sub>4a,4b</sub> 9.0 Hz, 1H, H<sub>4b</sub>), 4.49 (d, *J*<sub>4a,4b</sub> 9.0 Hz, 1H, H<sub>4a</sub>) (other peaks identical to **5.12a**, as reported above).

EIMS *m/z* 243 (M<sup>+</sup>, 0.3%), 228 (M<sup>+</sup>-CH<sub>3</sub>, 3), 225 (M<sup>+</sup>-H<sub>2</sub>O, 0.4), 212 (6), 211 (2), 172 (5), 171 (2), 160 (3), 159 (8), 158 (2), 154 (4), 144 (9), 143 (3), 128 (14), 127 (25), 117 (12), 116 (5), 111 (6), 110 (10), 109 (80), 102 (21), 86 (61), 85 (28), 84 (11), 83 (30), 82 (38), 81 (6), 71 (10), 70 (9), 69 (23), 68 (7), 67 (22), 58 (14), 57 (100), 56 (22), 55 (84).

**2,3-*trans*-2-(1-Hydroxy-6-methylheptyl)-3-(*t*-butyldimethylsilyloxymethyl) butanolides (6.11) and (6.12b)**



To a well-stirred solution of TBDMS protected A-factor (**4.35**) (100 mg, 0.28 mmol) in anhydrous methanol (10 mL)  $-10^\circ\text{C}$  was added sodium borohydride (9 mg, 0.24 mmol). After 5 minutes the mixture was allowed to warm to room temperature for another 40 minutes at which time no starting material was detected by TLC ( $\text{SiO}_2$ ; 15% EtOAc in hexane).

The reaction was quenched by the addition of phosphate buffer solution (pH 5.0, 3 mL) and the bulk of the methanol was removed from the resulting mixture on a rotary evaporator ( $20^\circ\text{C}$ ). After extraction with EtOAc (3 x 5 mL) the combined extracts were washed successively with water (5 mL) and saturated brine, then dried and evaporated to give an oily residue. Radial chromatography (1 mm  $\text{SiO}_2$ ; 1-3% MeOH in  $\text{CH}_2\text{Cl}_2$ ) allowed separation of the two 2,3-*trans* diastereomers.

The first eluted (**6.11**,  $R_f = 0.73$ ; 4% MeOH in  $\text{CH}_2\text{Cl}_2$ ) was isolated as a colourless oil in 65% yield.

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.70 (s, 6H,  $\text{SiCH}_3$ ), 0.85 (d,  $J = 6.6$  Hz, 6H, H12,13), 0.89 (s, 9H,  $^t\text{Bu}$ ), 1.12-1.23 (m, 2H, H10), 1.23-1.43 (m, 3H, H8a,9), 1.43-1.66 (m, 4H, H7, 8a,11), 2.60 (dd,  $J_{2,3} = 8.6$  Hz,  $J_{2,6} = 5.2$  Hz, 1H, H2), 2.64 (m, 1H, H3), *ca* 3.0 (d,  $J_{\text{OH},6} = 6$  Hz, 1H, OH), 3.66 (dd,  $J_{5b,3} = 4.8$  Hz,  $J_{5b,5a} = 11.5$  Hz, 1H, H5b), 3.69 (dd,  $J_{5a,3} = 4.9$  Hz,  $J_{5a,5b} = 11.5$  Hz, 1H, H5a), 3.84 (m, 1H, H6), 4.03 (dd,  $J_{4b,3} = 7.8$  Hz,  $J_{4b,4a} = 8.9$  Hz, 1H, H4b), 4.37 (dd,  $J_{4a,3} = 8.0$  Hz,  $J_{4a,4b} = 8.9$  Hz, 1H, H4a).

$^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ )  $\delta$  -5.9 ( $\text{SiCH}_3$ ), 17.9 (q,  $^t\text{Bu}$ ), 22.4 (C12, 13), 25.5 ( $^t\text{Bu}$ ), 25.6 (C8), 27.0 (C9), 27.7 (C11), 34.4 (C7), 38.7 (C10), 40.3 (C3), 47.2 (C2), 62.5 (C5), 68.7 (C4), 71.0 (C6), 178.2 (C1).

EIMS  $m/z$  301 ( $\text{M}^+ - \text{C}_4\text{H}_9$ , 0.4%), 283 (2.5), 173 (22), 155 (11), 145 (4), 143 (6), 115 (5), 95 (8), 89 (6), 85 (14), 82 (10), 75 (100), 73 (16), 69 (17), 59 (11), 57 (29), 56 (17), 55 (62).

CIMS  $m/z$  376 ( $\text{M} + \text{NH}_4^+$ , 3%), 359 ( $\text{M} + \text{H}^+$ , 85), 341 (70).

HRMS Found 301.1843;  $\text{C}_{15}\text{H}_{29}\text{O}_4\text{Si}$  ( $\text{M}^+ - \text{C}_4\text{H}_9$ ) requires 301.1835.

The second eluted diastereomer (**6.12b**,  $R_f = 0.52$ ; 4% MeOH in  $\text{CH}_2\text{Cl}_2$ ) isolated as a colourless oil in 25% yield.

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.60 (s, 6H,  $\text{SiCH}_3$ ), 0.86 (d,  $J = 6.6$  Hz, 6H, H12,13), 0.89 (s, 9H,  $^t\text{Bu}$ ), 1.12-1.23 (m, 2H, H10), 1.25-1.38 (m, 3H, H8b,9), 1.43-1.64 (m,



4H, H7,8a,11), *ca* 2.1 (m, 1H, OH), 2.60 (dd,  $J_{2,3} = 7$  Hz,  $J_{2,6} = 3.1$  Hz, 1H, H2), 2.82 (m, 1H, H3), 3.65 ('d',  $J = 5.1$  Hz, 2H, H5), 4.08 (dd,  $J_{4a,4b} = 8.9$  Hz,  $J_{4b,3} = 6.5$  Hz, 1H, H4b), 4.15 (m, 1H, H6), 4.36 (dd,  $J_{4a,4b} = 8.9$  Hz,  $J_{4a,3} = 8.7$  Hz, 1H, H4a).

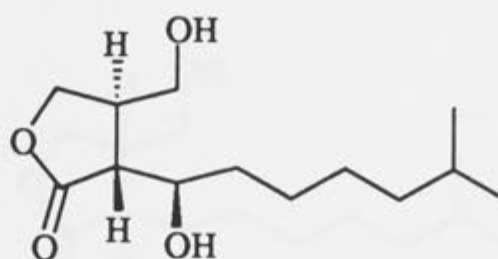
$^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ )  $\delta$  -5.9 ( $\text{SiCH}_3$ ), 17.9 (q, 'Bu), 22.4 (C12, 13), 25.5 ('Bu), 25.9 (C8), 27.0 (C9), 27.7 (C11), 34.7 (C7), 37.5 (C3), 38.7 (C10), 47.4 (C2), 63.1 (C5), 69.5 (C4), 70.6 (C6), 179.0 (C1).

EIMS  $m/z$  283 ( $\text{M}^+ - \text{C}_4\text{H}_9 - \text{H}_2\text{O}$ , 1%), 173 (12), 145 (3), 143 (4), 115 (4), 95 (8), 85 (14), 82 (12), 75 (100), 73 (16), 69 (20), 59 (12), 57 (36), 56 (24), 55 (68).

CIMS  $m/z$  376 ( $\text{M} + \text{NH}_4^+$ , 19%), 359 ( $\text{M} + \text{H}^+$ , 84), 341 (100).

HRMS Found 301.1843;  $\text{C}_{15}\text{H}_{29}\text{O}_4\text{Si}$  ( $\text{M}^+ - \text{C}_4\text{H}_9$ ) requires 301.1835.

## 2-(1-Hydroxy-6-methylheptyl)-3-(hydroxymethyl)butanolide (1.2) [i.e. Factor I]



1.2 factor-I

To an iced cooled (0°C) solution of the lower polarity TBDMS-protected butanolide diastereomer (isolated above) (**6.11**, 26 mg, 0.072 mmol) in THF (5 mL) was added and 1.0 M solution of tetra *n*-butylammonium fluoride in THF (0.27 mL, 0.27 mmol). After stirring for 35 min the mixture was allowed to warm to room temperature before being quenched with the addition of phosphate buffer solution (pH 7.0, 2 mL). After dichloromethane extraction (3 x 5 mL), the combined extracts were dried and evaporated to leave an oily residue which was purified by radial chromatography (1 mm SiO<sub>2</sub>, 1-3% MeOH in CH<sub>2</sub>Cl<sub>2</sub>). The title compound (**1.2**) was isolated as a colourless oil, (1.5 mg, 79%) and had NMR and mass spectral data in full agreement with factor-I **1.2**.<sup>26</sup>

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.85 (d, *J* = 6.6 Hz, 6H, H11,12), 1.10-1.23 (m, 2H, H10), 1.23-1.40 (m, 3H, H8b,9), 1.43-1.67 (m, 4H, H7,8a,11), 2.65 (dd, *J*<sub>2,6</sub> = 4.7 Hz, *J*<sub>2,3</sub> = 9.5 Hz, 1H, H2), 2.77 (m, 1H, H3), 3.67 (dd, *J*<sub>5a,5b</sub> = 10.7 Hz, *J*<sub>5a,3</sub> = 5.2 Hz, 1H, H5a), 3.97 (dd, *J*<sub>4b,4a</sub> = 8.9 Hz, *J*<sub>4b,3</sub> = 8.8 Hz, 1H, H4b), 4.01 (m, 1H, H6), 4.41 (dd, *J*<sub>4a,4b</sub> = 9.0 Hz, *J*<sub>4a,3</sub> = 8.2 Hz, 1H, H4a).

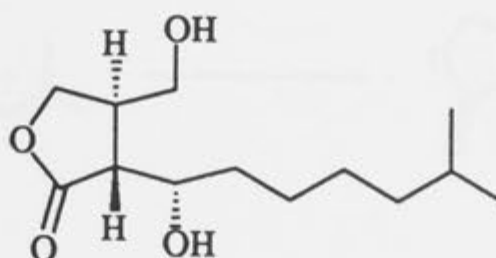
<sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ 22.4 (C12, 13), 25.9 (C8), 27.0 (C9), 27.7 (C11), 33.8 (C7), 38.7 (C10), 39.9 (C3), 49.1 (C2), 62.9 (C5), 68.3 (C4), 70.8 (C6), 177.7 (C1).

EIMS *m/z* 226 (M<sup>+</sup>-H<sub>2</sub>O, 0.7%), 195 (7), 171 (2), 153 (2), 145 (19), 139 (4), 129 (4), 127 (4), 116 (15), 111 (7), 95 (13), 85 (37), 69 (30), 67 (15), 56 (56), 55 (24), 54 (36), 42<sup>†</sup> (100).

<sup>†</sup> This value may have deeper significance as "The Answer to the Great Question of Life, the Universe, and Everything" (see "The Hitch Hiker's Guide to the Galaxy", Douglas Adams, Pan Books: London 1979, p 135).



**2-(1-Hydroxy-6-methylheptyl)-3-(hydroxymethyl)butanolide (1.5)** [i.e. Gräfe's factor from *S. bikiniensis* and *S. cyaneofuscatus*]



**1.5** Gräfe's factor

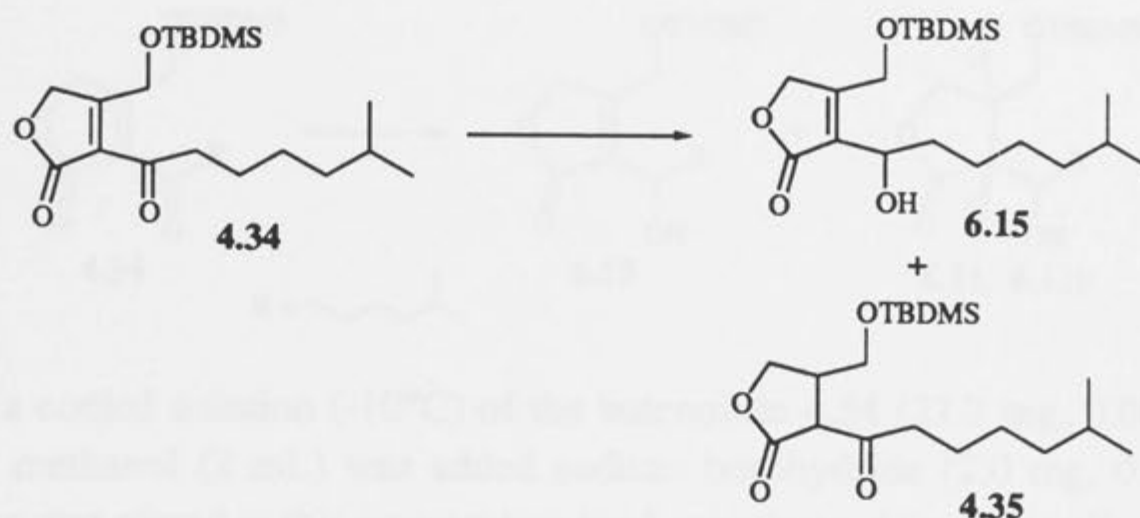
The higher polarity TBDMS-protected butanolide diastereomer (**6.12b**, 11.0 mg, 0.031 mmol), was deprotected using the same procedure as that described above for the less polar diastereomer **6.11**. After chromatography, the title compound was isolated as a colourless oil (7 mg, 93%) and displayed NMR and mass spectra in full agreement with the naturally derived compound.<sup>28</sup>

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.86 (d, *J* = 6.6 Hz, 6H, H12,13), 1.12-1.23 (m, 2H, H10), 1.23-1.38 (m, 3H, H8b,9), 1.43-1.68 (m, 4H, H7,8a,11), 2.56 (dd, *J*<sub>2,6</sub> = 3.7 Hz, *J*<sub>2,3</sub> = 7.4 Hz, 1H, H2), 2.86 (m, 1H, H3), 3.69 (dd, *J*<sub>5b,3</sub> = 6.2 Hz, *J*<sub>5b,5a</sub> = 10.6 Hz, 1H, H5b), 3.75 (dd, *J*<sub>5a,5b</sub> = 10.6 Hz, *J*<sub>5a,3</sub> = 5.6 Hz, 1H, H5a), 4.10 (dd, *J*<sub>4b,4a</sub> = 9.0 Hz, *J*<sub>4b,3</sub> = 6.8 Hz, 1H, H4b), 4.12 (m, 1H, H6), 4.42 (dd, *J*<sub>4a,3</sub> = 8.6 Hz, 1H, H4a).

<sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ 22.4 (C12, 13), 25.9 (C8), 27.0 (C9), 27.7 (C11), 34.7 (C7), 37.9 (C3), 38.7 (C10), 48.0 (C2), 63.3 (C5), 69.4 (C4), 70.8 (C6), 180.3 (C1).

EIMS *m/z* 226 (M<sup>+</sup>-H<sub>2</sub>O, 0.5%), 195 (6), 171 (3), 153 (2), 145 (22), 139 (3), 129 (4), 127 (5), 116 (19), 111 (9), 95 (16), 85 (39), 69 (38), 67 (20), 56 (65), 55 (34), 54 (43), 42 (100).

## 1,2-Reduction of the Butenolide 4.34



A solution of TBDMS-protected butenolide (**4.34**, 64 mg, 0.18 mmol) in anhydrous methanol (4 mL) was cooled to 0°C under an atmosphere of argon before the addition of a solution of anhydrous cerium trichloride (45 mg, 0.18 mmol) in methanol (2 mL). Sodium borohydride was then added (6.8 mg, 0.18 mmol as a solid) and the mixture was stirred for 5 mins. The reaction was quenched with the addition of phosphate buffer solution (pH 5.0, 1 mL) and water (2 mL). Extraction with CH<sub>2</sub>Cl<sub>2</sub> (4 x 4 mL), successive washing of the combined extracts with water (5 mL) and brine (5 mL) followed by drying and evaporation afforded an oily residue which was subjected to radial chromatography (2 mm SiO<sub>2</sub>, 0-2% MeOH in CH<sub>2</sub>Cl<sub>2</sub>).

After a fore-fraction which was found to contain 8.4 mg (13%) of a 4:1 mixture of TBDMS-A factor (**4.35**) and unreacted butenolide (**4.34**), the 1,2-reduction product (**6.15**) was isolated as a colourless oil (45 mg, 70%).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.10 (s, 6H, SiCH<sub>3</sub>), 0.85 (d, *J* = 6.6 Hz, 6H, H<sub>12,13</sub>), 0.91 (s, 9H, <sup>t</sup>Bu), 1.10-1.21 (m, 2H, H<sub>10</sub>), 1.24-1.37 (m, 3H, H<sub>8b,9</sub>), 1.37-1.46 (m, 1H, H<sub>8a</sub>), 1.46-1.58 (m, 1H, H<sub>11</sub>), 1.58-1.83 (m, 2H, H<sub>7</sub>), 2.70 (br s, 1H, OH), 4.51 (t, *J* = 5 Hz, 1H, H<sub>6</sub>), 4.66 (d, *J*<sub>5a,5b</sub> = 16.8 Hz, 1H, H<sub>5b</sub>), 4.73 (d, *J*<sub>5a,5b</sub> = 16.8 Hz, 1H, H<sub>5a</sub>), 4.78 (s, 2H, H<sub>4</sub>).

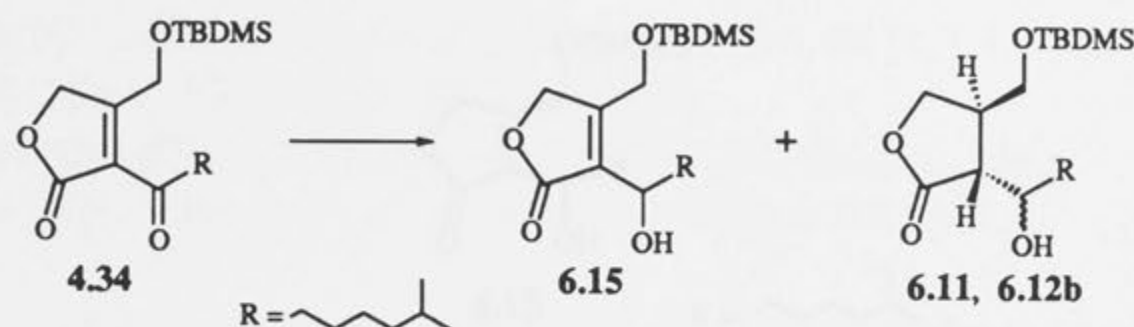
<sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ -5.6 (SiCH<sub>3</sub>), 18.2 (q, <sup>t</sup>Bu), 22.6 (C<sub>12,13</sub>), 25.7 (<sup>t</sup>Bu), 25.8 (C<sub>8</sub>), 27.1 (C<sub>9</sub>), 27.9 (C<sub>11</sub>), 36.7 (C<sub>7</sub>), 38.9 (C<sub>10</sub>), 58.8 (C<sub>5</sub>), 67.3 (C<sub>6</sub>), 70.5 (C<sub>4</sub>), 127.7 (C<sub>2</sub>), 160.3 (C<sub>3</sub>), 173.7 (C<sub>1</sub>).

EIMS *m/z* 299 (M<sup>+</sup>-C<sub>4</sub>H<sub>9</sub>, 2%), 257 (M<sup>+</sup>-C<sub>7</sub>H<sub>15</sub>, 8), 207 (2), 161 (2), 151 (4), 137 (5), 125 (4), 123 (4), 119 (4), 111 (6), 109 (8), 107 (6), 105 (9), 97 (8), 95 (6), 93 (7), 91 (6), 89 (6), 86 (15), 84 (25), 77 (12), 75 (100), 73 (51), 69 (17), 59 (11), 57 (35), 55 (27).

HRMS Found 299.1679; C<sub>15</sub>H<sub>27</sub>O<sub>4</sub>Si (M<sup>+</sup>-C<sub>4</sub>H<sub>9</sub>) requires 299.1679.

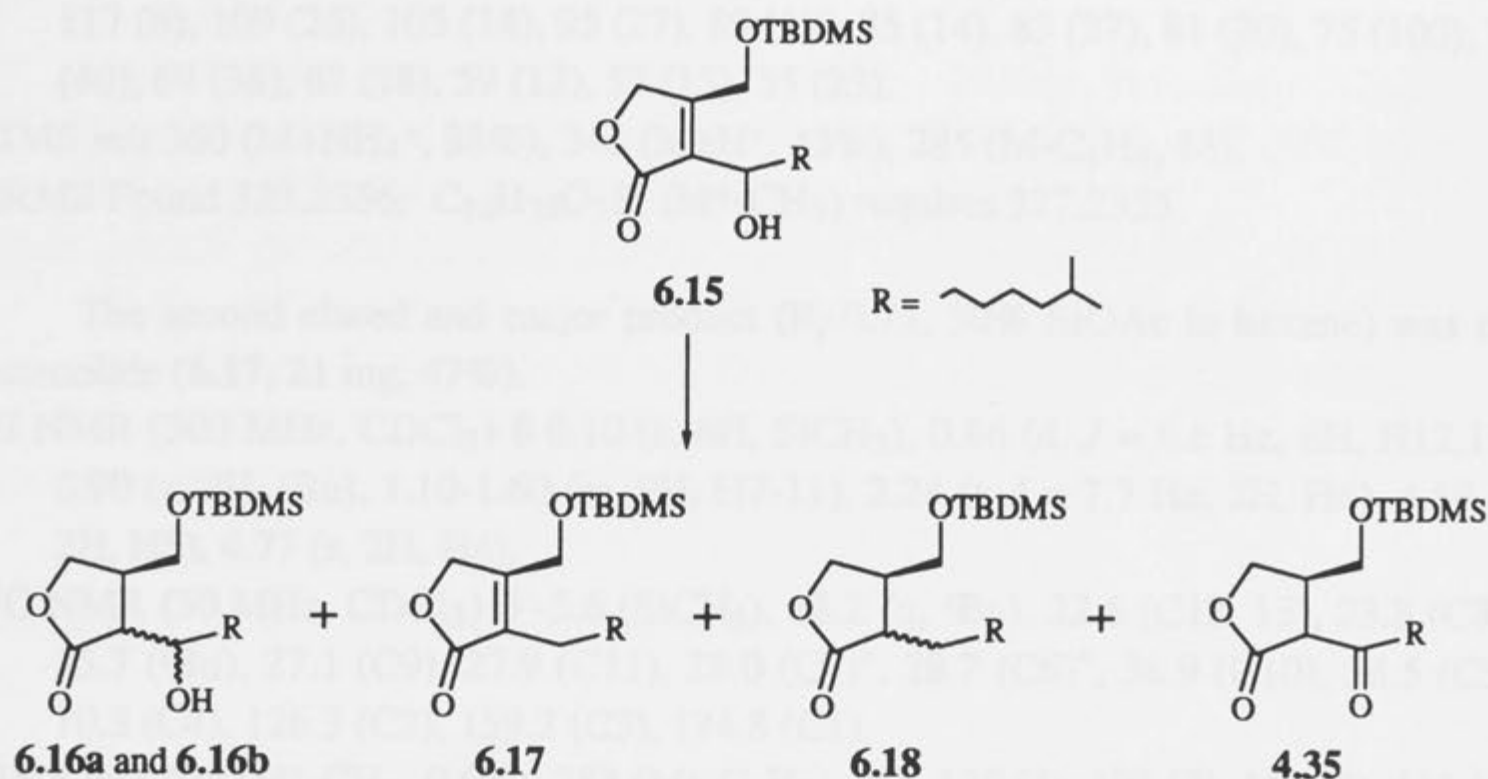


## Reduction of the Butenolide 4.34 with Sodium Borohydride in Methanol



To a cooled solution ( $-10^\circ\text{C}$ ) of the butenolide **4.34** (21.7 mg, 0.061 mmol) in anhydrous methanol (2 mL) was added sodium borohydride (2.0 mg, 0.053 mmol). The mixture was stirred at this temperature for 5 minutes and then was allowed to warm room temperature a over 40 minute period. After quenching with the addition of phosphate buffer (pH 5, 1 mL) and water (5mL), the mixture was extracted with  $\text{CH}_2\text{Cl}_2$  (3 x 2mL), dried and evaporated to afford a pale yellow oil.  $^1\text{H}$  NMR spectroscopy of the product revealed the presence of a mixture of the 2,3-*trans* 6-hydroxybutanolides **6.11** and **6.12b**, and a small amount of the 1,2-reduction product **6.15** (ratio *ca* 16:4:1). After radial chromatography (1 mm  $\text{SiO}_2$  / 1-4% MeOH in  $\text{CH}_2\text{Cl}_2$ ) the first eluted compound was the butenolide **6.15** (0.9 mg, 4%), followed by the 6-hydroxybutanolides **6.11** (12.5 mg, 58%) and the other C6 epimeric compound **6.12b** (3.2 mg, 15%).  $^1\text{H}$  NMR data and TLC characteristics for all three products were identical to those reported above.

## Catalytic Hydrogenation of the 6-Hydroxybutenolide (6.15)



## METHOD A: USING PALLADIUM-ON-CARBON CATALYSIS

The 6-hydroxybutenolide (**6.15**, 47 mg, 0.132 mmol), in methanol solution (20 mL) was hydrogenated at room pressure and temperature in the presence of 10% palladium-on-carbon (3 mg). After 3 hours, one equivalent of hydrogen had been absorbed and the catalyst was removed by filtration through a bed of TLC grade silica gel (0.5 cm). The oily residue remaining after removal of the solvent was subjected to careful radial chromatography (1 mm SiO<sub>2</sub>; 10-20% EtOAc in hexane) which enabled the isolation of four products, followed by a small amount of unreacted starting material.

The first eluted compound (*R<sub>f</sub>* 0.73, 30% EtOAc in hexane) was 2-(6'-methylheptyl)-3-(*t*-butyldimethylsilyloxymethyl)butanolide (**6.18**, 7.8 mg, 17%) as a 70 : 30 mixture of diastereomers.

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) **6.18a** (major diastereomer) δ 0.06 (s, 6H, SiCH<sub>3</sub>), 0.87 (d, *J* = 6.6 Hz, 6H, H12,13), 0.89 (s, 9H, <sup>*t*</sup>Bu), 1.05-1.90 (m, 11H, H6-11), 2.50-2.62 (m, 2H, H2,3), 3.60 (dd, *J*<sub>5a,5b</sub> = 10.3 Hz, *J*<sub>5b,3</sub> = 5.7 Hz, 1H, H5b), 3.68 (dd, *J*<sub>5a,5b</sub> = 10.3 Hz, *J*<sub>5a,3</sub> = 3.3 Hz, 1H, H5a), 4.23 (dd, *J*<sub>4b,4a</sub> = 9.0 Hz, *J*<sub>4b,3</sub> = 5.5 Hz, 1H, H4b), 4.30 (dd, *J*<sub>4a,4b</sub> = 9.0 Hz, *J*<sub>4a,3</sub> = 1.2 Hz, 1H, H4a).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) **6.18b** (minor diastereomer) δ 0.06 (s, 6H, SiCH<sub>3</sub>), 0.87 (d, *J* = 6.6 Hz, 6H, H12,13), 0.89 (s, 9H, <sup>*t*</sup>Bu), 1.05-1.90 (m, 11H, H6-11), 2.38-2.49 (m, 2H, H2,3), 3.57-3.72 (m, 2H, H5a,5b), 4.07 (dd, *J*<sub>4b,4a</sub> = 9.1 Hz, *J*<sub>4b,3</sub> = 7.1 Hz, 1H, H4b), 4.33 (dd, *J*<sub>4a,4b</sub> = 9.1 Hz, *J*<sub>4a,3</sub> = 7.6 Hz, 1H, H4a).

<sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) **6.18a** (major diastereomer) δ -5.6 (SiCH<sub>3</sub>), 18.2 (q, <sup>*t*</sup>Bu), 22.6 (C12,13), 24.8 (C8), 25.6 (<sup>*t*</sup>Bu), 27.1 (C9), 27.9 (C11), 28.1 (C7)\*, 29.8 (C6)\*, 38.9 (C10), 39.8 (C3)\*\*, 41.4 (C2)\*\*, 58.5 (C5), 70.3 (C4), 174.8 (C1). Signal to



noise ratio did not allow unambiguous identification of the  $^{13}\text{C}$  peaks of the minor diastereomer.

EIMS  $m/z$  327 ( $\text{M}^+-\text{CH}_3$ , 1%), 285 ( $\text{M}^+-\text{C}_4\text{H}_9$ , 11), 257 (6), 197 (5), 173 (6), 145 (5), 117 (6), 109 (26), 105 (14), 95 (27), 89 (18), 85 (14), 83 (27), 81 (20), 75 (100), 73 (40), 69 (38), 67 (18), 59 (13), 57 (15), 55 (23).

CIMS  $m/z$  360 ( $\text{M}+\text{NH}_4^+$ , 38%), 343 ( $\text{M}+\text{H}^+$ , 43%), 285 ( $\text{M}-\text{C}_4\text{H}_9$ , 18).

HRMS Found 327.2356;  $\text{C}_{18}\text{H}_{35}\text{O}_3\text{Si}$  ( $\text{M}^+-\text{CH}_3$ ) requires 327.2355.

The second eluted and major product ( $R_f$  0.71, 30% EtOAc in hexane) was the butenolide (**6.17**, 21 mg, 47%).

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.10 (s, 6H,  $\text{SiCH}_3$ ), 0.86 (d,  $J = 6.6$  Hz, 6H, H12,13), 0.90 (s, 9H,  $t\text{-Bu}$ ), 1.10–1.60 (m, 9H, H7–11), 2.24 (t,  $J = 7.7$  Hz, 2H, H6), 4.58 (s, 2H, H5), 4.77 (s, 2H, H4).

$^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ )  $\delta$  -5.6 ( $\text{SiCH}_3$ ), 18.2 (q,  $t\text{-Bu}$ ), 22.6 (C12, 13), 23.8 (C8)\*, 25.7 ( $t\text{-Bu}$ ), 27.1 (C9), 27.9 (C11), 28.0 (C7)\*, 29.7 (C6)\*, 38.9 (C10), 58.5 (C5), 70.3 (C4), 126.3 (C2), 159.2 (C3), 174.8 (C1).

EIMS  $m/z$  325 ( $\text{M}^+-\text{CH}_3$ , 0.6%), 283 ( $\text{M}^+-\text{C}_4\text{H}_9$ , 25), 129 (3), 123 (3), 117 (3), 111 (5), 109 (11), 107 (4), 105 (6), 97 (6), 95 (11), 89 (8), 85 (6), 83 (13), 81 (11), 79 (6), 77 (9), 75 (100), 73 (42), 69 (27), 67 (11), 59 (15), 57 (19), 55 (22).

CIMS  $m/z$  358 ( $\text{M}+\text{NH}_4^+$ , 38%), 341 ( $\text{M}+\text{H}^+$ , 33), 283 ( $\text{M}^+-\text{C}_4\text{H}_9$ , 17).

HRMS Found 325.5199;  $\text{C}_{18}\text{H}_{33}\text{O}_3\text{Si}$  ( $\text{M}^+-\text{CH}_3$ ) requires 325.5199.

Third eluted ( $R_f$  0.53, 30% EtOAc in hexane) was 2,3-*cis*-2-(1'-hydroxy-6'-methylheptyl)-3-(*t*-butyldimethylsilyloxymethyl)butanolide (**6.16a**, 1.9 mg, 4%).

$^1\text{H}$  NMR (300 MHz, acid-free  $\text{CDCl}_3$ )  $\delta$  0.07 (s, 6H,  $\text{SiCH}_3$ ), 0.87 (d,  $J = 6.5$  Hz, 6H, H12,13), 0.89 (s, 9H,  $t\text{-Bu}$ ), 1.12–1.26 (m, 2H, H10), 1.26–1.47 (m, 4H, H8,9), 1.47–1.65 (m, 3H, H7,11), 2.58 (t,  $J = 8$  Hz, 1H, H2), 2.61 (m, 1H, H3), 3.68 (dd,  $J_{5b,5a} = 10.3$  Hz,  $J_{5b,3} = 3.8$  Hz, 1H, H5b), 3.71 (dd,  $J_{5a,5b} = 10.3$  Hz,  $J_{5a,3} = 3.5$  Hz, 1H, H5a), 3.99 (m, 1H, H6), 4.16 (d,  $J_{6,\text{OH}} = 2.0$  Hz, 1H, OH), 4.28 (dd,  $J_{4b,4a} = 9.1$  Hz,  $J_{4b,3} = 5.2$  Hz, 1H, H4b), 4.32 (dd,  $J_{4a,4b} = 9.0$  Hz,  $J_{4a,3} = 2.1$  Hz, 1H, H4a).

$^{13}\text{C}$  NMR (50 MHz, acid-free  $\text{CDCl}_3$ )  $\delta$  -5.7 ( $\text{SiCH}_3$ ), 18.1 (q,  $t\text{-Bu}$ ), 22.6 (C12,13), 25.5 (C8), 25.6 ( $t\text{-Bu}$ ), 27.3 (C9), 27.9 (C11), 35.5 (C7), 38.9 (C10), 39.7 (C3), 46.2 (C2), 61.1 (C5), 67.8 (C4), 70.0 (C6), 179.0 (C1).

EIMS  $m/z$  325 ( $\text{M}^+-\text{CH}_3$ , 0.3%), 301 ( $\text{M}^+-\text{C}_4\text{H}_9$ , 1), 283 (3), 173 (11), 155 (6), 145 (3), 143 (3), 127 (6), 115 (5), 109 (6), 107 (4), 105 (8), 95 (12), 89 (12), 85 (15), 81 (11), 75 (100), 73 (35), 69 (25), 67 (10), 59 (15), 57 (30), 56 (13), 55 (65).

CIMS  $m/z$  359 ( $\text{M}+\text{H}^+$ , 100%), 341 (30), 301 (22).

HRMS Found 301.1834;  $\text{C}_{15}\text{H}_{29}\text{SiO}_4$  ( $\text{M}^+-\text{C}_4\text{H}_9$ ) requires 301.1835.

The fourth product eluted ( $R_f$  0.44, 30% EtOAc in hexane) was the alternative C6 epimer of 2,3-*cis*-2-(1'-hydroxy-6'-methylheptyl)-3-(*t*-butyldimethylsilyloxymethyl)-butanolide (**6.16b**, 2.0 mg, 4%).

$^1\text{H}$  NMR (300 MHz, acid-free  $\text{CDCl}_3$ )  $\delta$  0.10 (s, 6H,  $\text{SiCH}_3$ ), 0.87 (d,  $J = 6.6$  Hz, 6H, H12,13), 0.91 (s, 9H,  $t\text{-Bu}$ ), 1.12–1.25 (m, 2H, H10), 1.25–1.44 (m, 3H, H8b,9), 1.47–1.66 (m, 3H, H7b,8a,11), 1.97 (m, 1H, H7a), 2.74 (t,  $J = 7.2$  Hz, 1H, H2),



2.83 (m, 1H, H3), 3.07 (d,  $J_{6,\text{OH}} = 4.3$  Hz, 1H, OH), 3.76 (dd,  $J_{5b,5a} = 10.6$  Hz,  $J_{5b,3} = 5.6$  Hz, 1H, H5b), 3.87 (dd,  $J_{5a,5b} = 10.6$  Hz,  $J_{5a,3} = 7$  Hz, 1H, H5a), 4.03 (m, 1H, H6), 4.19 (dd,  $J_{4b,4a} = 9.3$  Hz,  $J_{4b,3} = 2.0$  Hz, 1H, H4b), 4.23 (dd,  $J_{4a,4b} = 9.3$  Hz,  $J_{4a,3} = 4.8$  Hz, 1H, H4a).

$^{13}\text{C}$  NMR (50 MHz, acid-free  $\text{CDCl}_3$ )  $\delta$  -5.6 ( $\text{SiCH}_3$ ), 18.1 (q,  $t\text{Bu}$ ), 22.6 (C12,13), 25.7 ( $t\text{Bu}$ ), 26.0 (C8), 27.2 (C9), 27.9 (C11), 34.7 (C7), 39.0 (C10), 40.7 (C3), 48.9 (C2), 62.3 (C5), 68.2 (C4), 69.1 (C6), 176.0 (C1).

EIMS  $m/z$  325 ( $\text{M}^+ - \text{CH}_3$ , 0.3%), 301 ( $\text{M}^+ - \text{C}_4\text{H}_9$ , 1), 283 (3), 173 (11), 155 (6), 145 (3), 143 (3), 127 (6), 115 (5), 109 (6), 107 (4), 105 (8), 95 (12), 89 (12), 85 (15), 81 (11), 75 (100), 73 (35), 69 (25), 67 (10), 59 (15), 57 (30), 56 (13), 55 (65).

CIMS  $m/z$  359 ( $\text{M} + \text{H}^+$ , 100%), 341 (30), 301 (22).

HRMS Found 301.1834;  $\text{C}_{15}\text{H}_{29}\text{SiO}_4$  ( $\text{M}^+ - \text{C}_4\text{H}_9$ ) requires 301.1835.

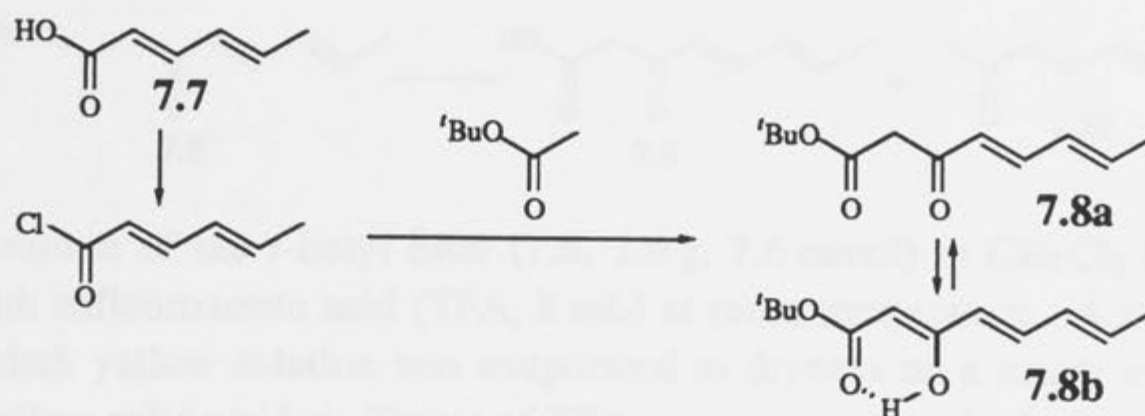
#### METHOD B: USING PLATINUM CATALYSIS

The 6-hydroxy butenolide (**6.15**, 20.2 mg, 0.057 mmol) in benzene (5 mL) was hydrogenated over platinum black (3 mg) for 20 hours at room temperature and pressure. After workup and chromatography described above the following products were collected; (in order of elution) TBDMS-protected A-factor (**4.35**, 3.0 mg, 15%); butanolide (**6.18**, 1.8 mg, 10%); butenolide (**6.17**, 0.7 mg, 4%); 2,3-*cis*-6-hydroxy butanolides (**6.16a**, 4.0 mg, 20%) and (**6.16b**, 4.0 mg, 20%) followed by unreacted (**6.15**, 0.8 mg, 4%). (Spectroscopic data as described above).

#### METHOD C: USING RHODIUM-ON-ALUMINA

Hydrogenation of (**6.15**, 35.6 mg, 0.10 mmol) in methanol (20 mL) over 5% rhodium-on-alumina (5 mg) until hydrogen absorption ceased (4 hours) gave a mixture of products which was purified as described above. In order of elution, the products collected were; TBDMS-protected A-factor (**4.35**, 11.5 mg, 32%); butanolide (**6.18**, 4.8 mg, 14%); 2,3-*cis*-6-hydroxy butanolide (**6.16a**, 5.7 mg, 16%); 2,3-*trans*-6-hydroxy butanolide (**6.11**, 0.7 mg, 2%); 2,3-*trans*-6-hydroxy butanolide (**6.12**, *ca* 0.5 mg, *ca* 2%) and 2,3-*cis*-6-hydroxy butanolide (**6.16b**, 5.3 mg, 15%). (Spectroscopic data for all compounds described above).



*t*-Butyl (*E,E*)-3-oxoocta-4,6-dienoate (7.8)

A solution of lithium diisopropylamide (LDA) was prepared by the dropwise addition of *n*-butyllithium solution (1.55 M in hexane, 64 mL, 0.1 mol) to a cooled (-50°C) solution of diisopropylamine (10.1 g, 0.1 mol) in anhydrous THF (100 mL) under an atmosphere of argon. After stirring for 10 minutes at -50°C, *t*-butylacetate (5.8 g, 0.05 mol) was slowly (2 mm) introduced. The mixture was stirred for a further 10 minutes then cooled to -78°C before sorbyl chloride<sup>231</sup> (6.5 g, 0.05 mol) was added over 10 minutes. During the addition the solution became yellow and the temperature rose to -50°C. After 10 minutes at this temperature, the reaction was quenched with the addition of dilute hydrochloric acid (20% w/v, 30 mL).

Following extraction with ether (3 x 50 mL) the combined organic extracts were washed sequentially with saturated aqueous sodium bicarbonate solution (2 x 30 mL) and saturated brine (30 mL). Drying and evaporation of the solvent gave a yellow oil which was purified by fractional distillation under reduced pressure. The required *t*-butyl ester (7.8, 5.6 g, 53%) was isolated as a yellow oil, boiling at 90-92°C / 0.3 mm Hg. Upon cooling the oil solidified and a sample was recrystallised from hexane to give pale yellow needles (mp 47-51°C).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 1.44 (s, 9H, <sup>t</sup>Bu), 1.48 (s, enol <sup>t</sup>Bu), 1.82 (d, *J* = 6.5 Hz, enol H8), 1.87 (d, *J* = 5.1 Hz, 3H, H8), 3.47 (s, 2H H2), 4.93 (s, enol H2), 5.75 (d, *J*<sub>4,5</sub> = 15.3 Hz, enol H4), 6.02 (m, enol H7), 6.11 (d, *J*<sub>4,5</sub> = 15.4 Hz, 1H, H4), 5.94-6.32 (m, 2H, H6,7+enol H6), 6.98 (dd, *J*<sub>5,4</sub> = 15.2 Hz, *J*<sub>5,6</sub> = 10.6 Hz, enol H5), 7.14 (dd, *J*<sub>5,4</sub> = 15.7 Hz, *J*<sub>5,6</sub> = 8.9 Hz, 1H, H5), [enol content 23%].

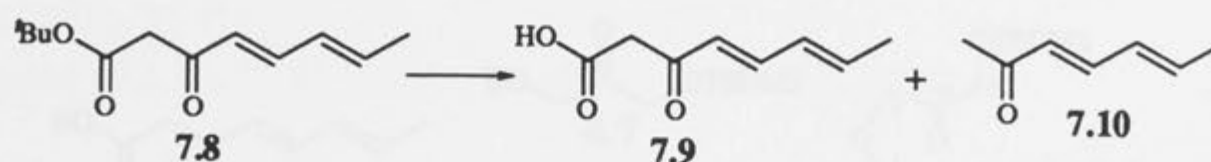
<sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ 18.5 (enol C8), 18.7 (C8), 27.8 (<sup>t</sup>Bu), 28.2 (<sup>t</sup>Bu), 48.5 (C2), 80.6 (enol 9, <sup>t</sup>Bu), 81.5 (q, <sup>t</sup>Bu), 92.4 (enol C2), 123.0 (enol C4), 126.7 (C4), 130.0 (C6), 130.5 (C6 enol), 136.0 (enol C7)\*, 136.6 (enol C5)\*, 141.3 (C7), 144.6 (C5), 166.6 (C1), 169.0 (enol C1)\*, 172.5 (enol C3)\*, 192.7 (C3).

EIMS *m/z* 210 (M<sup>+</sup>, 5%), 195 (M<sup>+</sup>-15, 0.4), 154 (M<sup>+</sup>-C<sub>4</sub>H<sub>8</sub>, 16), 139 (29), 137 (10), 136 (15), 121 (11), 109 (17), 108 (11), 95 (52), 80 (21), 69 (8), 67 (30), 65 (10), 59 (18), 57 (100).

Analysis: Found C 68.2, H 8.6; C<sub>12</sub>H<sub>18</sub>O<sub>3</sub> requires C 68.5, H 8.6.



## 3-Oxo-octa-4,6-dienoic acid (7.9)



A solution of the *t*-butyl ester (7.8, 1.6 g, 7.6 mmol) in  $\text{CH}_2\text{Cl}_2$  (12 mL) was treated with trifluoroacetic acid (TFA, 8 mL) at room temperature. After 1 hour, the resulting dark yellow solution was evaporated to dryness on a rotary evaporator, to afford a yellow solid residue. Traces of TFA were removed under high vacuum before the solid was recrystallised from ether to give the  $\beta$ -keto acid (7.9) as fine, pale yellow needles (0.59 g, 50%), melting at 97–99°C. The product could be sublimed at *ca* 90°C / 0.03 mm Hg but the resulting product contained *ca* 10% of the decarboxylation product, hepta-3,5-diene-2-one (7.10)<sup>232</sup> by  $^1\text{H}$  NMR.

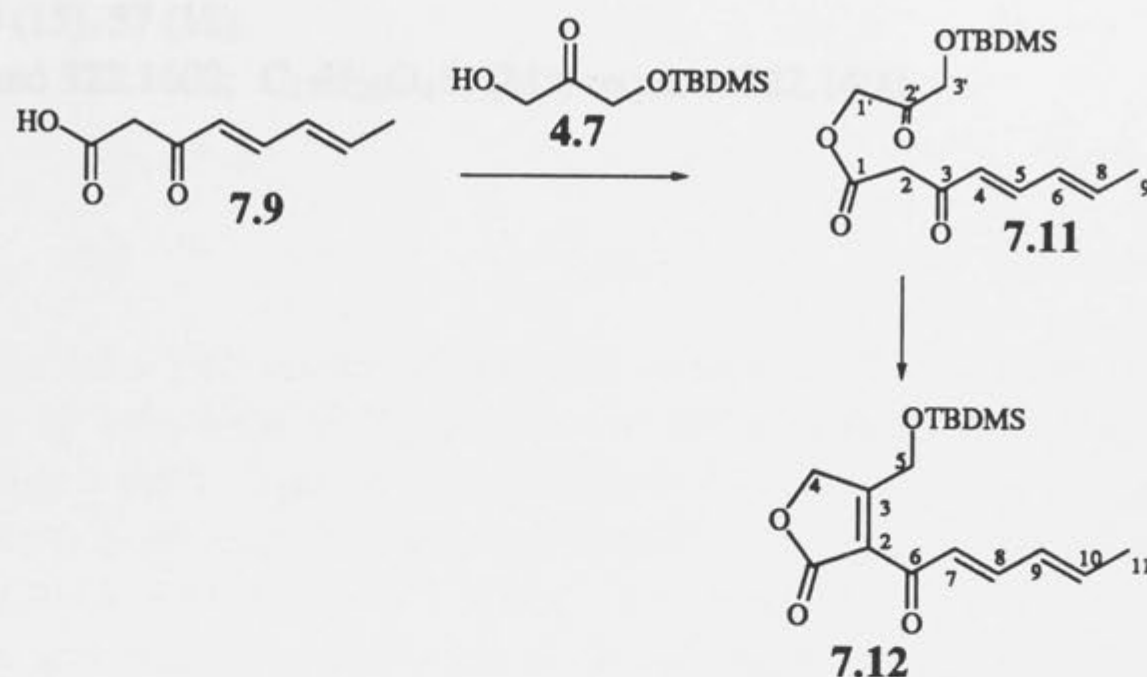
$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  1.86 (d,  $J = 6.0$  Hz, enol H8), 1.92 (d,  $J = 6.3$  Hz, 3H, H8), 3.66 (s, 2H, H2), 5.07 (s, enol H2), 5.82 (d,  $J = 15.3$  Hz, enol H4), 6.14 (d,  $J = 15.7$  Hz, 1H, H4), 6.02–6.43 (m, 2H, H6,7), 7.08 (dd,  $J = 10.5$  Hz,  $J = 15.5$  Hz, enol H5), 7.26 (dd,  $J_{4,5} = 15.7$  Hz,  $J_{5,6} = 10.5$  Hz, 1H, H5), 11.61 (s, enol OH), [enol content 38%].

$^{13}\text{C}$  NMR (50 MHz,  $\text{CD}_3\text{COCD}_3$ )  $\delta$  18.0 (C8)\*, 18.2 (enol C8)\*, 46.2 (C2), 90.2 (enol C2), 123.3 (enol C4), 127.5 (C4), 130.5 (enol C6)\*, 130.8 (C6)\*, 136.8 (enol C7)\*, 137.6 (enol C5)\*, 141.3 (C7), 144.7 (C5), 168.4 (C1), 171.2 (enol C1)\*, 174.5 (enol C3)\*, 193.0 (C3).

EIMS  $m/z$  154 ( $\text{M}^+$ , 2%), 139 ( $\text{M}^+ - \text{CH}_3$ , 5), 136 ( $\text{M}^+ - \text{H}_2\text{O}$ , 2), 121 (2), 110 (38), 109 (9), 96 (7), 95 (100), 91 (7), 81 (6), 79 (5), 77 (16), 69 (4), 68 (5), 67 (71), 66 (9), 65 (18), 63 (6), 55 (12), 53 (12), 51 (11).

HRMS Found 154.0630;  $\text{C}_8\text{H}_{10}\text{O}_3$  requires 154.0650.



3-(*t*-Butyldimethylsilyloxymethyl)-2-(hexa-2,4-dienoyl)but-2-enolide (7.12)

3-Oxo-octa-4,6-dienoic acid (7.9, 154 mg, 1.0 mmol) was esterified with 1-hydroxy-3-*t*-butyldimethylsilyloxypropan-2-one (4.7, 234 mg, 1.15 mmol) using dicyclohexylcarbodiimide (DCC, 216 mg, 1.05 mmol) catalysed by DMAP (15 mg) and DMAP hydrochloride (5 mg) in  $\text{CH}_2\text{Cl}_2$  (5 mL), following the procedure described above for the preparation of 3'-(*t*-Butyldimethylsilyloxy)-2'-oxopropyl 3-oxononanoate (4.6).

After aqueous work-up the crude product mixture (425 mg) was shown by  $^1\text{H}$  NMR analysis, using an internal standard (triphenylmethane), to contain *ca* 180 mg (*ca* 53%) of the required  $\beta$ -ketoester intermediate (7.11):

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.10 (s, 6H,  $\text{SiCH}_3$ ), 0.91 (s, 9H,  $\text{'Bu}$ ), 1.84 (d,  $J = 6.5$  Hz, enol H8), 1.88 (d,  $J = 5.6$  Hz, 3H, H8), 3.71 (s, 2H, H2), 4.27 (s, enol H3')\*, 4.29 (s, 2H, H3')\*, 5.01 (s, enol H1')\*\*, 5.02 (s, 2H, H1')\*\*, 5.17 (s, enol H2), 5.81 (d,  $J = 15.4$  Hz, enol H4), 6.04 (d,  $J_{4,5} = 15.0$  Hz, 1H, H4), 5.98-6.37 (m, 2H, H6,7), 7.04 (dd,  $J = 10.4$  Hz,  $J = 15.1$  Hz, enol H4), 7.21 (dd,  $J_{5,4} = 15.5$  Hz,  $J_{5,6} = 9.6$  Hz, 1H, H5), [enol content 50%].

During subsequent radial chromatography (2 batches; 2 mm  $\text{SiO}_2$ ; 10-30% EtOAc in hexane), the intermediate ester cyclised to afford the title butenolide (7.12, 135 mg) as the first eluted compound. Later bands contained the unreacted silyloxypropanone (4.7, 125 mg) and hepta-3,5-dien-2-one (7.10)<sup>232</sup> (50 mg).

The butenolide product (7.12) appeared as an orange oil (*ca* 80% pure by  $^1\text{H}$  NMR; 34% yield over 2 steps) and was used in the subsequent reduction without further purification. A sample was recrystallised from pentane to give orange needles, mp 82-88°C.

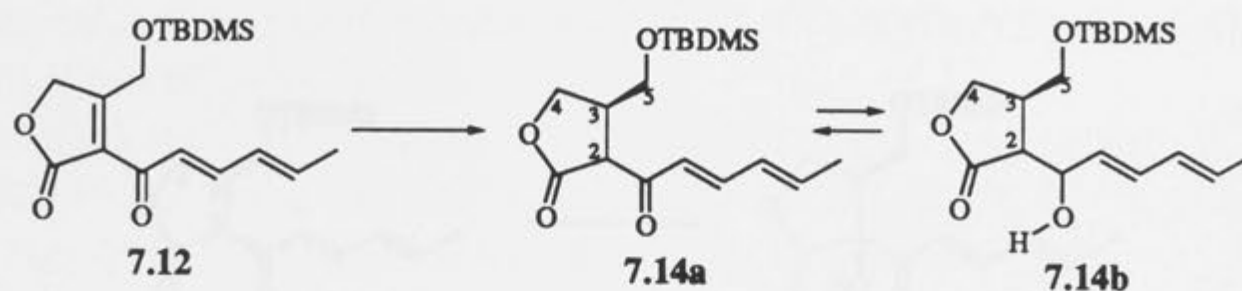
$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.11 (s, 6H,  $\text{SiCH}_3$ ), 0.91 (s, 9H,  $\text{'Bu}$ ), 1.90 (d,  $J = 5.5$  Hz, 3H, H11), 5.00 (s, 2H, H4)\*, 5.12 (s, 2H, H5)\*, 6.18-6.47 (m, 2H, H9,10), 7.26 (d,  $J_{7,8} = 15.3$  Hz, 1H, H7), 7.38 (dd,  $J_{8,7} = 15.1$  Hz,  $J_{8,9} = 9.2$  Hz, 1H, H8).

$^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ )  $\delta$  -5.6 ( $\text{SiCH}_3$ ), 18.1 (q,  $\text{'Bu}$ ), 25.7 ( $\text{'Bu}$ ), 62.0 (C5), 70.3 (C4), 122.7 (C2), 124.0 (C7), 130.8 (C9), 142.8 (C10), 145.8 (C8), 170.8 (C3)\*, 181.7 (C1)\*, 184.9 (C6).

EIMS  $m/z$  322 ( $M^+$ , 3%), 266 (5), 265 ( $M^+ - C_4H_9$ , 21), 235 (5), 219 (3), 211 (2), 209 (2), 207 (2), 196 (3), 191 (3), 190 (3), 169 (3), 147 (3), 146 (3), 145 (4), 129 (4), 117 (5), 115 (5), 105 (5), 103 (3), 95 (45), 91 (7), 77 (12), 75 (100), 73 (52), 67 (27), 65 (9), 59 (15), 57 (18).

HRMS Found 322.1602;  $C_{17}H_{26}O_4Si$  ( $M^+$ ) requires 322.1600.



3-(*t*-Butyldimethylsilyloxymethyl)-2-(hexa-2,4-dienoyl)butanolide (7.14)

To a cooled ( $-5^{\circ}\text{C}$ ) solution of the crude butenolide (7.12, 88 mg, *ca* 80% pure, 0.22 mmol) in anhydrous THF (5 mL) was added sodium borohydride (25 mg, 0.66 mmol) as a solid. The mixture was allowed to warm to room temperature for 30 min. before quenching with aqueous hydrochloric acid (0.2 M, 10 mL). After dichloromethane extraction (3 x 7 mL), the combined extracts were washed sequentially with water (2 x 5 mL) and phosphate buffer (pH 7.0, 10 mL), then dried and evaporated to give a dark oily residue. Radial chromatography (1 mm,  $\text{SiO}_2$ ; 15–30% EtOAc in hexane) afforded the saturated lactone (7.14;  $R_f = 0.41$ , 25% EtOAc in hexane) as an orange oil (44 mg, 65%).

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.04 (s, 6H,  $\text{SiCH}_3$ ), 0.88 (s, 9H,  $t\text{-Bu}$ ), 1.87 (d,  $J = 6.2$  Hz, enol H11), 1.89 (d,  $J = 5.6$ , 1H, H11), 3.15–3.31 (m, 1H, H3+enol H3), 3.53 (dd,  $J_{5b,5a} = 10.0$  Hz,  $J_{5b,3} = 8.1$  Hz, enol H5b), 3.64 (dd,  $J_{5a,5b} = 9.8$  Hz,  $J_{5a,3} = 5.3$  Hz, enol H5a), 3.66 (‘d’,  $J = 4.6$  Hz, 2H, H5a,5b), 3.85 (d,  $J_{2,3} = 6.3$  Hz, 1H, H2), 4.17 (dd,  $J_{4b,4a} = 8.8$  Hz,  $J_{4b,3} = 6.0$  Hz, 1H, H4b), 4.31–4.39 (m, enol H4a,4b), 4.43 (dd,  $J_{4a,4b} = 8.8$  Hz,  $J_{4a,3} = 7.8$  Hz, 1H, H4a), 5.94 (d,  $J = 15.2$  Hz, enol H7), 6.04–6.25 (m, enol H9,10), 6.26–6.33 (m, 2H, H9,10), 6.40 (d,  $J = 15.4$  Hz, 1H, H7), 7.11 (dd,  $J_{8,7} = 15.2$  Hz,  $J_{8,9} = 10.3$  Hz, enol H8), 7.25–7.35 (m, 1H, H8), 10.95 (s, enol OH) [content of enol 7.14b; 55%].

$^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ )  $\delta$  -5.5 ( $\text{SiCH}_3$ ), 18.2 (q,  $t\text{-Bu}$ ), 18.8 (enol C11)\*, 19.0 (c11)\*, 25.7 ( $t\text{-Bu}$ )\*, 25.8 (enol  $t\text{-Bu}$ )\*, 39.5 (enol C3)\*, 39.9 (C3)\*, 52.9 (C2), 62.1 (C5), 65.4 (enol C5), 69.3 (C4), 70.0 (enol C4), 97.2 (enol C2), 119.6 (enol C7), 125.6 (C7), 130.2 (C9), 130.6 (enol C9), 138.0 (enol C10)\*, 139.1 (enol C8)\*, 142.7 (C10), 146.0 (C8), 163.6 (C1), 172.9 (enol C1)\*, 176.8 (enol C3)\*, 191.8 (C3).

EIMS  $m/z$  324 ( $\text{M}^+$ , 0.3%), 309 ( $\text{M}^+ - \text{CH}_3$ , 1), 267 ( $\text{M}^+ - \text{C}_4\text{H}_9$ , 30), 179 (9), 173 (4), 119 (3), 117 (4), 115 (5), 111 (3), 105 (6), 96 (7), 95 (100), 91 (7), 89 (14), 85 (3), 83 (4), 77 (7), 75 (54), 73 (39), 67 (28), 59 (12), 57 (12).

HRMS Found 324.1758;  $\text{C}_{17}\text{H}_{28}\text{O}_4\text{Si}$  ( $\text{M}^+$ ) requires 324.1757.



**(*E,E*)-3-(*t*-Butyldimethylsilyloxymethyl)-2-(hexa-2,4-dienoyl)-2-hydroxybutanolide (7.21a and 7.21b)**



To a solution at room temperature of the butanolide (**7.14**, 30 mg, 0.092 mmol) in anhydrous THF (3 mL) was added sodium hydride (60% oil dispersion, 4.1 mg, 0.10 mmol). Ultrasonication of the reaction mixture for 3 min. gave a clear, yellow solution to which was added *m*-chloroperbenzoic acid (*m*-CPBA, *ca* 80% pure, 44 mg, 0.20 mmol). After further ultrasonication (3 min.) the solution was almost completely decolourised and subsequent TLC analysis revealed no starting material. After 15 mins. the oxidation was quenched by the addition of 2-propanol (0.3 mL) and water (2 mL). Extraction with dichloromethane (3 x 5 mL), followed by sequential washing of the combined extracts [saturated NaHCO<sub>3(aq)</sub> (10 mL); H<sub>2</sub>O (10 mL) and phosphate buffer (pH 7.0, 5 mL)], drying and evaporation, gave a pale yellow oil which was subjected to radial chromatography (1 mm, SiO<sub>2</sub>; 10-30% EtOAc in hexane) which allowed isolation of two compounds.

First eluted (*R<sub>f</sub>* = 0.38, 25% EtOAc in hexane) was the β-hydroxy diastereomer of the title hydroxydiene (**7.21a**) as a colourless oil (9.3 mg, 29%). Relative geometry of the products was determined by NOE studies as described in Chapter 7.

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.05 (s, 6H, SiCH<sub>3</sub>), 0.86 (s, 9H, <sup>*t*</sup>Bu), 1.90 (d, *J* = 5.9 Hz, 3H, H11), 2.96 (m, 1H, H3), 3.79 (dd, *J*<sub>5b,3</sub> = 5.2 Hz, *J*<sub>5b,5a</sub> = 10.5 Hz, 1H, H5b), 3.84 (dd, *J*<sub>5a,3</sub> = 4.9 Hz, *J*<sub>5a,5b</sub> = 10.4 Hz, 1H, H5a), 4.21 (s, 1H, OH), 4.27 (dd, *J*<sub>4b,3</sub> = 4.9 Hz, *J*<sub>4b,4a</sub> = 8.9 Hz, 1H, H4b), 4.56 (dd, *J*<sub>4a,3</sub> = 7.4 Hz, *J*<sub>4a,4b</sub> = 9.0 Hz, 1H, H4a), 6.20-6.43 (m, 2H, H9,10), 6.54 (d, *J*<sub>7,8</sub> = 15.1 Hz, 1H, H7), 7.43 (dd, *J*<sub>8,9</sub> = 10.1 Hz, *J*<sub>8,7</sub> = 15.0 Hz, 1H, H8).

<sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ -5.7 (SiCH<sub>3</sub>), 18.0 (q, <sup>*t*</sup>Bu), 19.0 (C11), 25.6 (<sup>*t*</sup>Bu), 44.6 (C3), 60.5 (C5), 69.3 (C4), 81.1 (C2), 119.6 (C7), 130.3 (C9), 143.9 (C10), 147.3 (C8), 173.9 (C1), 194.2 (C6).

EIMS *m/z* 283 (M<sup>+</sup>-C<sub>4</sub>H<sub>9</sub>, 3%), 253 (4), 237 (2), 225 (10), 157 (5), 96 (7), 95 (100), 89 (3), 75 (20), 73 (13), 67 (23), 65 (3), 59 (4), 57 (3), 55 (5).

HRMS Found 283.1003; C<sub>13</sub>H<sub>19</sub>O<sub>5</sub>Si (M<sup>+</sup>-C<sub>4</sub>H<sub>9</sub>) requires 283.1002.

The second eluted compound (*R<sub>f</sub>* = 0.27, 25% EtOAc in hexane) was the α-hydroxy diastereomer of the title compound (**7.21b**) as a colourless oil (16.5 mg, 52%).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.00 (s, 6H, SiCH<sub>3</sub>), 0.84 (s, 9H, <sup>*t*</sup>Bu), 1.91 (d, *J* = 6.7 Hz, 3H, H11), 2.98 (m, 1H, H3), 3.67 (dd, *J*<sub>5b,5a</sub> = 10.4 Hz, *J*<sub>5b,3</sub> = 6.8 Hz, 1H, H5b), 3.78 (dd, *J*<sub>5a,5b</sub> = 10.5 Hz, *J*<sub>5a,3</sub> = 5.9 Hz, 1H, H5a), 4.12 (s, 1H, OH), 4.31 (dd, *J*<sub>4b,4a</sub> = 10.5 Hz, *J*<sub>4b,3</sub> = 5.9 Hz, 1H, H4b), 4.54 (dd, *J*<sub>4a,4b</sub> = 9.0 Hz, *J*<sub>4a,3</sub> = 8.7



Hz, 1H, H4a), 6.17-6.45 (m, 2H, H9,10), 6.41 (d,  $J_{7,8} = 15.2$  Hz, 1H, H7), 7.39 (dd,  $J_{8,7} = 15.2$  Hz,  $J_{8,9} = 10.7$  Hz, 1H, H8).

$^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ )  $\delta$  -5.7 (SiCH<sub>3</sub>), 18.2 (q, 'Bu), 19.1 (C11), 25.7 ('Bu), 50.3 (C3), 59.2 (C5), 67.9 (C4), 82.0 (C2), 120.5 (C7), 130.2 (C9), 144.6 (C10), 147.0 (C8), 174.5 (C1), 194.4 (C6).

EIMS  $m/z$  283 ( $\text{M}^+ - \text{C}_4\text{H}_9$ , 7%), 253 (1), 225 (9), 157 (3), 115 (3), 105 (3), 96 (7), 95 (100), 89 (3), 77 (3), 75 (28), 73 (17), 69 (3), 67 (24), 65 (3), 59 (5), 57 (4), 55 (7).

HRMS Found 283.1003;  $\text{C}_{13}\text{H}_{19}\text{O}_5\text{Si}$  ( $\text{M}^+ - \text{C}_4\text{H}_9$ ) requires 283.1002.

**(2*R*\*,3*S*\*)-3-(*t*-Butyldimethylsilyloxymethyl)-2-[(*E*)-3-(3-methyloxiranyl)-1-oxoprop-2-enyl]-2-hydroxy-butanolide (7.25)**



To a  $\text{CDCl}_3$  (1.5 mL) solution of the diene **7.21b** (9.3 mg, 0.027 mmol) in an NMR tube containing a single activated molecular sieve (4Å), was added *m*-CPBA (*ca* 80% pure, 6.3 mg, *ca* 0.03 mmol) and the mixture was allowed to stand at room temperature with periodic  $^1\text{H}$  NMR analysis. After 16 hours, about 30% of the diene **7.21b** had reacted as judged by comparative integration of the emerging new methyl doublets between  $\delta$  1.2 and 1.8 and the diminishing methyl doublet of the starting dienone at  $\delta$  1.9. A further quantity of *m*-CPBA (*ca* 80% pure, 12.0 mg, *ca* 0.054 mmol) was then added and the reaction was monitored for 29 hours more, by which time only about 10% of the starting material remained and the reaction mixture had become more complex. Isopropanol (0.3 mL) and water (1.5 mL) were added, followed by extraction with  $\text{CH}_2\text{Cl}_2$ , then sequential washing of the extracts with saturated sodium bicarbonate solution (3 x 1 mL) and phosphate buffer solution (pH 7, 1 mL). Drying and evaporation afforded an oil which was purified by radial chromatography (1 mm  $\text{SiO}_2$  / 10-30% EtOAc in hexane). First eluted was some unreacted **7.21b** (0.7 mg, 8%) followed by the mono-epoxides **7.25** (2.4 mg, 25%) and then a complex mixture of more polar compounds.

**7.25** (1:1 diastereomeric mixture), ( $R_f$  = 0.13, 25% EtOAc in hexane)

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.02 (s, 6H,  $\text{SiCH}_3$ ), 0.86 (s, 9H,  $^t\text{Bu}$ ), 1.40 (d,  $J$  = 5.2 Hz, 3H, H11), 2.90-3.06 (m, 2H, H3,10), 3.22 (m, 1H, H9), 3.73 (d,  $J$  = 5.7 Hz, 2H, H5a,5b), 3.94 (s, 1H, OH), 4.33 (2 x dd,  $J_{4b,4a}$  = 10.7, 10.7 Hz,  $J_{4b,3}$  = 9.1, 9.1 Hz, 1H, H4b), 4.52 (2 x t,  $J$  = 8.8, 8.8 Hz, 1H, H4a), 6.80-6.86 (m, 2H, H7, H8).

$^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ )  $\delta$  -5.7 ( $\text{SiCH}_3$ ), 17.5 (2 x C11), 18.2 (q,  $^t\text{Bu}$ ), 25.7 ( $^t\text{Bu}$ ), 50.6 (2 x C3), 57.3, 57.4 (2 x C9), 57.9, 58.1 (2 x C10), 58.7, 58.8 (2 x C5), 67.4 (2 x C4), 82.2 (2 x C2), 124.8, 125.0 (2 x C7), 146.6, 146.7 (2 x C8), 174.2 (2 x C1), 194.1 (2 x C6).

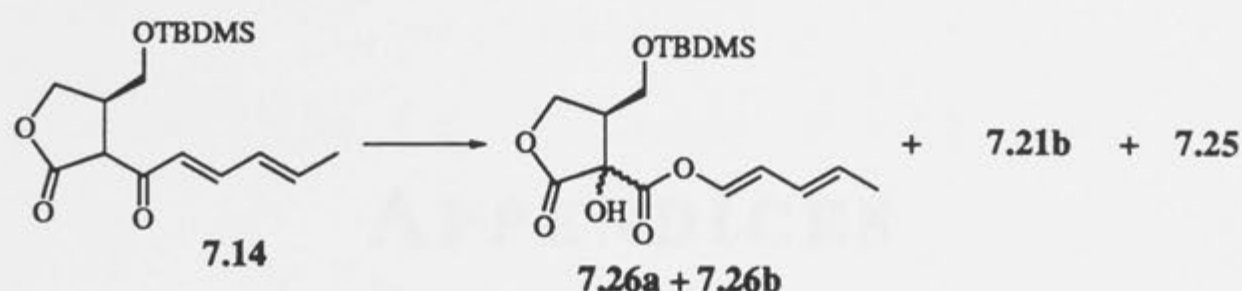
EIMS  $m/z$  299 ( $\text{M}^+ - \text{C}_4\text{H}_9$ , 0.3%), 281 (1), 241 (1), 225 (2), 223 (2), 211 (2), 157 (8), 143 (3), 131 (3), 129 (3), 115 (6), 111 (16), 105 (8), 101 (6), 99 (4), 97 (3), 95 (12), 91 (3), 89 (10), 85 (5), 84 (7), 83 (74), 77 (9), 76 (7), 75 (100), 74 (5), 73 (52), 59 (15), 57 (19), 55 (51).

CIMS  $m/z$  374 ( $\text{M} + \text{NH}_4^+$ , 50%), 357 ( $\text{M} + \text{H}^+$ , 31), 341 (100), 339 (59).

HRMS Found 299.0950;  $\text{C}_{13}\text{H}_{19}\text{O}_6\text{Si}$  ( $\text{M}^+ - \text{C}_4\text{H}_9$ ) requires 299.0951.



## Baeyer-Villiger Oxidation of 7.14



In the same manner as described above for the  $\alpha$ -hydroxylation of butanolide 7.14, the diene (7.14, 10 mg, 0.031 mmol) in anhydrous THF (1 mL) was oxidised by the sequential addition of sodium hydride (60% oil dispersion, 1.4 mg, 0.034 mmol) and *m*-chloroperbenzoic acid (*m*-CPBA, 21 mg, 0.10 mmol, 3.2 equiv.). After 16 hours of room temperature, a further quantity of *m*-CPBA was added (6.5 mg, 0.031 mmol, 1.0 equiv.) and stirring was continued for a further 13 hours. After workup (described above) the three major components of the crude reaction mixture were isolated by radial chromatography (1 mm SiO<sub>2</sub>, 10-30% EtOAc in hexane).

After a small initial fraction containing an impurity derived from *m*-CPBA, two diastereomers of the butyrolactone ester (7.26a and 7.26b) were isolated as colourless oils (1.0 mg, 9% and 1.4 mg, 13% respectively).

**7.26a** ( $R_f$  = 0.36, 25% EtOAc in hexane):

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.05 (s, 6H, SiCH<sub>3</sub>), 0.86 (s, 9H, <sup>t</sup>Bu), 1.78 (d,  $J$  = 6.8 Hz, 3H, H5'), 3.10 (m, 1H, H3), 3.77 (dd,  $J_{5b,5a}$  = 10.0 Hz,  $J_{5b,3}$  = 5.5 Hz, 1H, H5b), 3.78 (s, 1H, OH), 3.85 (dd,  $J_{5a,5b}$  = 10.3 Hz,  $J_{5a,3}$  = 6.5 Hz, 1H, H5a), 4.29 (dd,  $J_{4b,4a}$  = 8.7 Hz,  $J_{4b,3}$  = 6.3 Hz, 1H, H4b), 4.56 (dd,  $J_{4a,4b}$  = 8.7 Hz,  $J_{4a,3}$  = 7.3 Hz), 5.68-5.83 (m, 1H, H4'), 5.96 (m, 1H, H3'), 6.13 (dd,  $J_{2',3'}$  = 11.3 Hz,  $J_{2',1'}$  = 12.3 Hz, 1H, H2'), 7.26 (d,  $J_{1',2'}$  = 12.1 Hz, 1H, H1').

EIMS  $m/z$  356 (M<sup>+</sup>, 0.3%), 299 (M<sup>+</sup>-C<sub>4</sub>H<sub>7</sub>, 1), 255 (1), 241 (5), 217 (5), 199 (6), 187 (15), 159 (5), 158 (12), 157 (88), 131 (4), 129 (7), 115 (11), 113 (5), 105 (3), 101 (3), 99 (6), 89 (25), 85 (12), 84 (94), 83 (11), 76 (7), 75 (100), 74 (8), 73 (74), 67 (46), 59 (30), 58 (17), 57 (27), 56 (21), 55 (79), 53 (15).

HRMS Found 356.1664; C<sub>17</sub>H<sub>28</sub>O<sub>6</sub>Si (M<sup>+</sup>) requires 356.1665.

**7.26b** ( $R_f$  = 0.26, 25% EtOAc in hexane):

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.03 (d,  $J$  = 1.2 Hz, 6H, SiCH<sub>3</sub>), 0.86 (s, 9H, <sup>t</sup>Bu), 1.78 (d,  $J$  = 6.6 Hz, 3H, H5'), 3.00 (m, 1H, H3), 3.72 (s, 1H, OH), 3.79 (m, 2H, H5a,5b), 4.36 (dd,  $J_{4b,4a}$  = 8.9 Hz,  $J_{4b,3}$  = 10.9 Hz, 1H, H4b), 4.51 (dd,  $J_{4a,4b}$  = 8.8 Hz,  $J_{4a,3}$  = 8.6 Hz, 1H, H4a), 5.67-5.84 (m, 1H, H4'), 5.95 (m, 1H, H3'), 6.11 (dd,  $J_{2',3'}$  = 10.7 Hz,  $J_{2',1'}$  = 12.1 Hz, 1H, H2'), 7.25 (d,  $J_{1',2'}$  = 12.1 Hz, 1H, H1').

EIMS  $m/z$  299 (M<sup>+</sup>-C<sub>4</sub>H<sub>9</sub>, 4%), 274 (1), 273 (8), 257 (5), 243 (2), 242 (2), 241 (6), 215 (4), 199 (3), 198 (4), 187 (3), 159 (3), 158 (4), 157 (27), 141 (4), 113 (4), 101 (3), 99 (5), 95 (6), 89 (14), 85 (7), 84 (28), 83 (7), 77 (6), 76 (5), 75 (73), 74 (9), 73 (100), 69 (6), 67 (44), 59 (20), 57 (16), 55 (43).

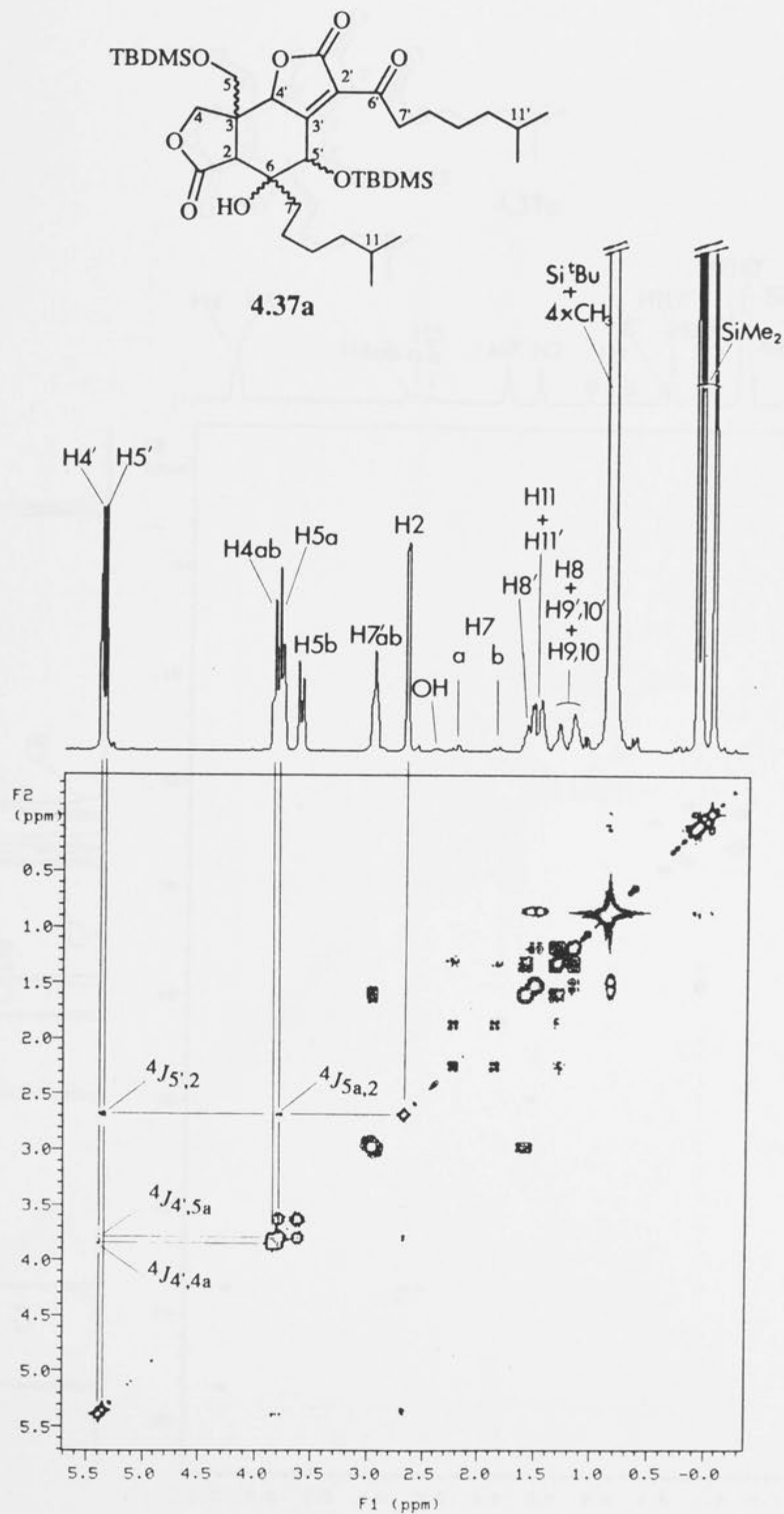
HRMS Found 299.0950; C<sub>13</sub>H<sub>19</sub>O<sub>6</sub>Si (M<sup>+</sup>-C<sub>4</sub>H<sub>9</sub>) requires 299.0951.

The third and final compound isolated was the previously isolated diene (7.21b, 2.8 mg, 27%) (spectroscopic data above).



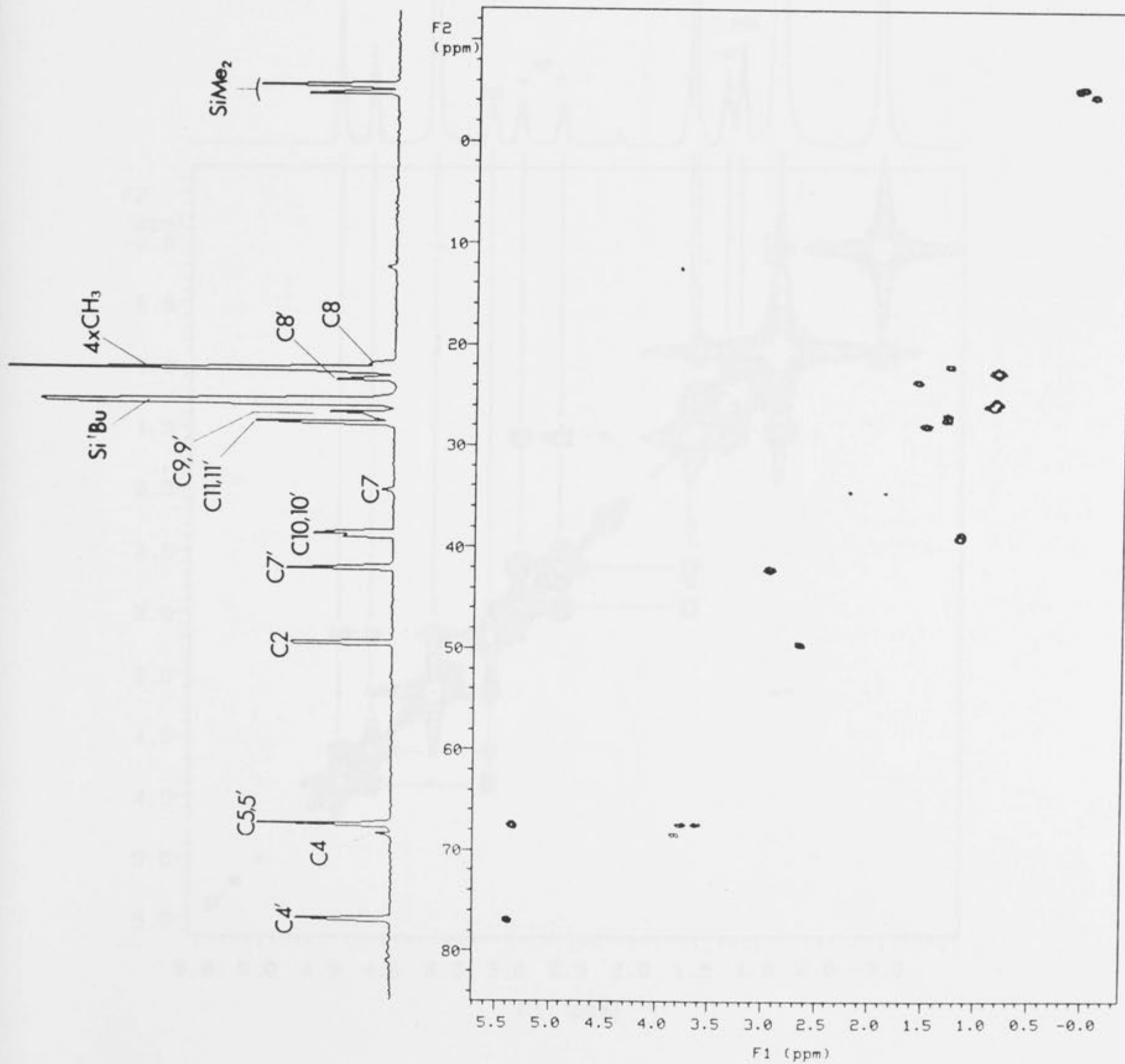
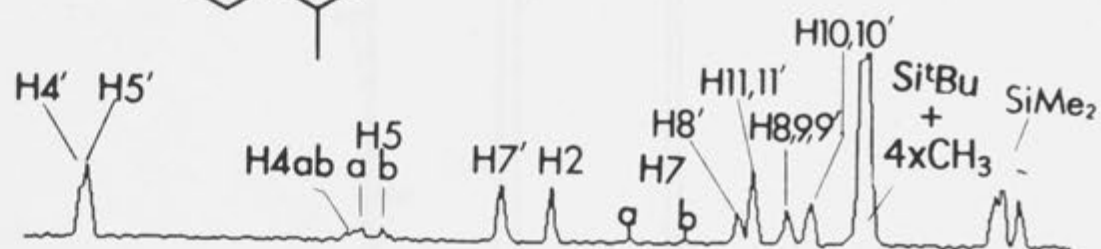
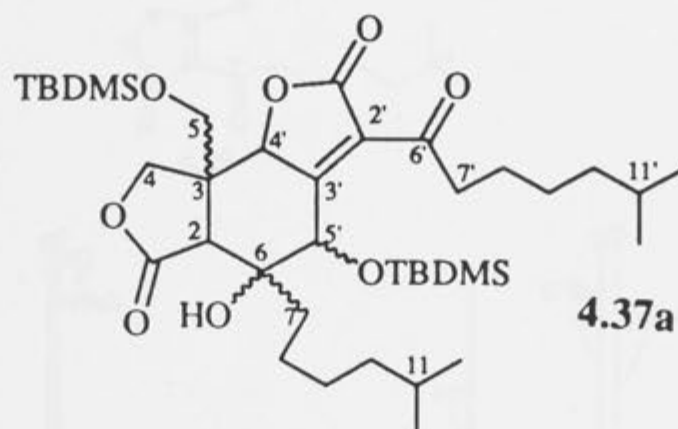


## Appendix Ai

 $^1\text{H}$ - $^1\text{H}$  COSY NMR Spectrum of Dimer (4.37a)

Appendix Aii

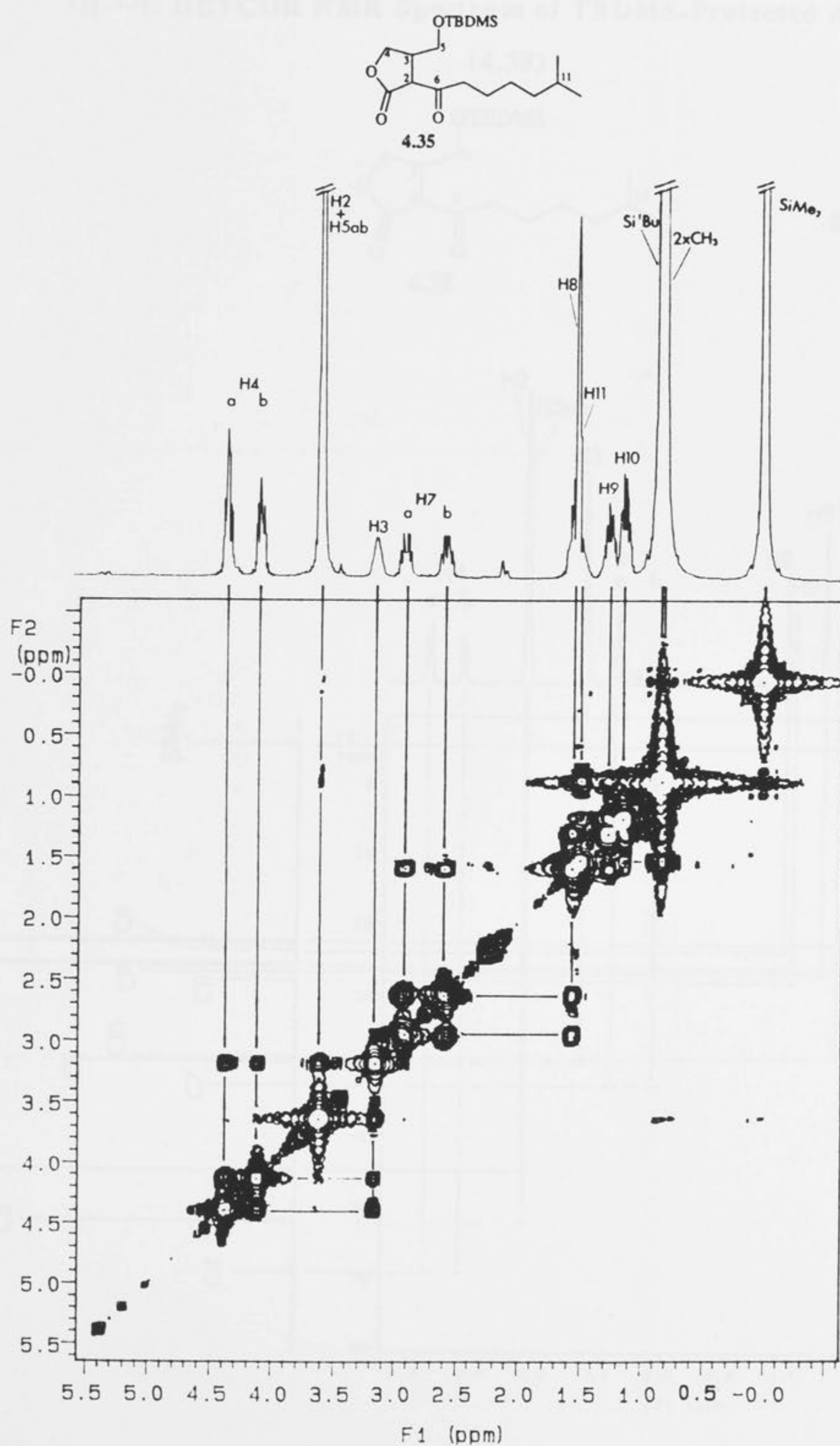
$^1\text{H}$ - $^{13}\text{C}$  HETCOR NMR Spectrum of Dimer (4.37a)





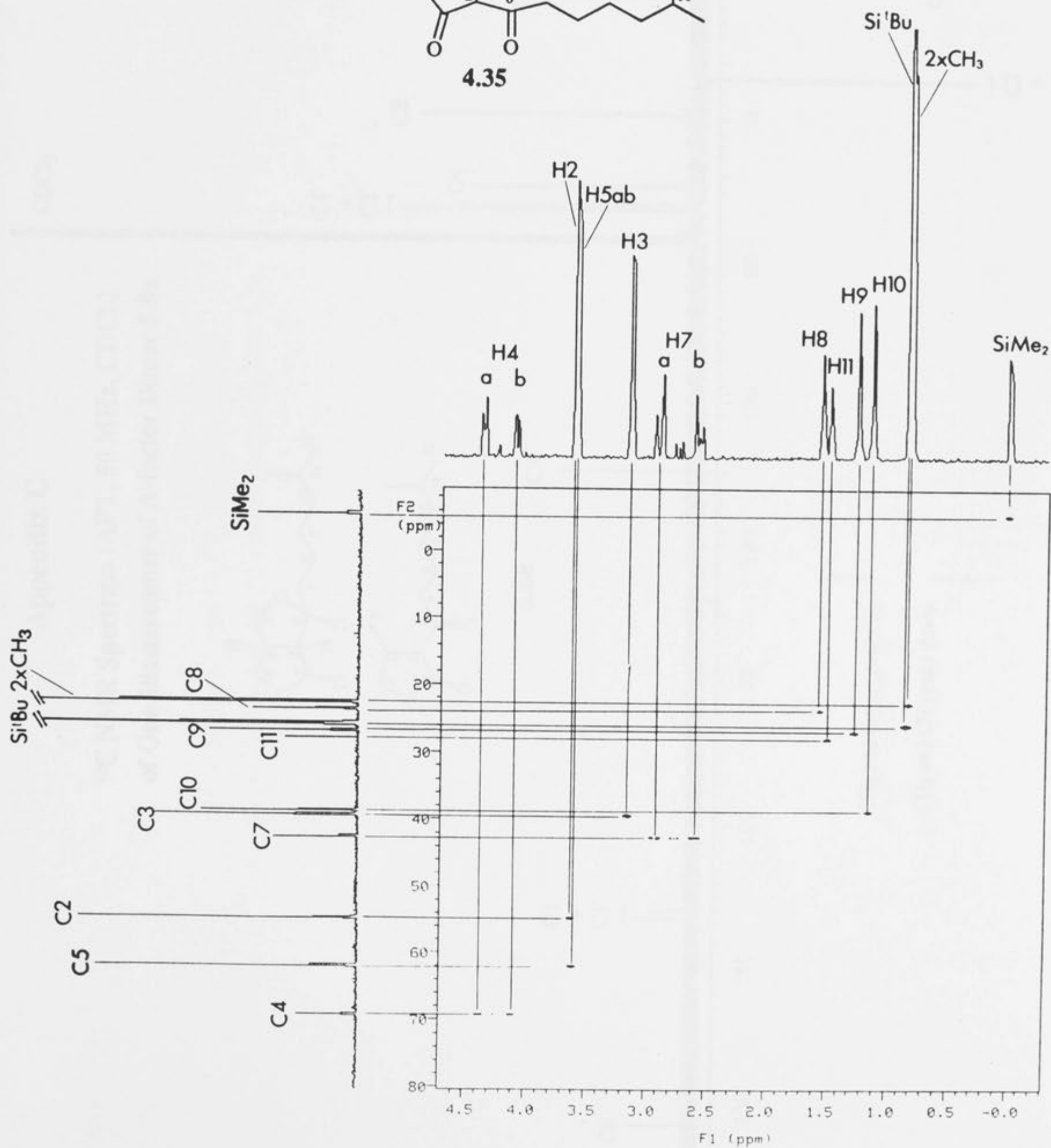
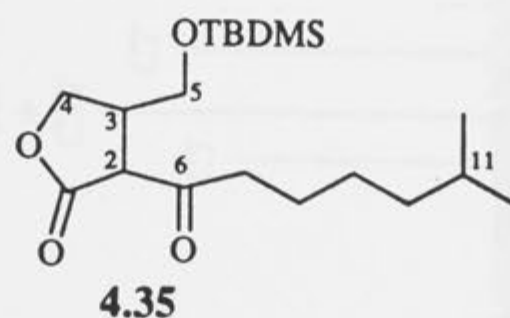
Appendix B i

<sup>1</sup>H-<sup>1</sup>H COSY NMR Spectrum of TBDMS-Protected A-Factor (4.35)



Appendix Bii

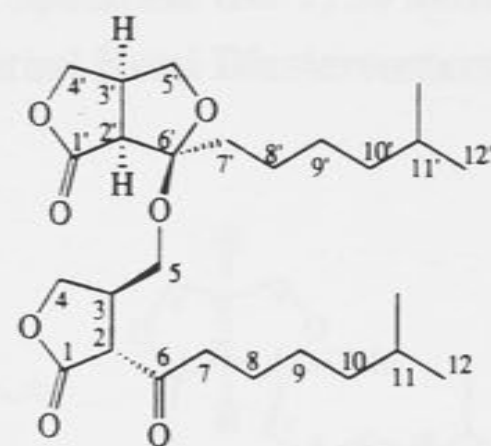
$^1\text{H}$ - $^{13}\text{C}$  HETCOR NMR Spectrum of TBDMS-Protected A-Factor  
(4.35)



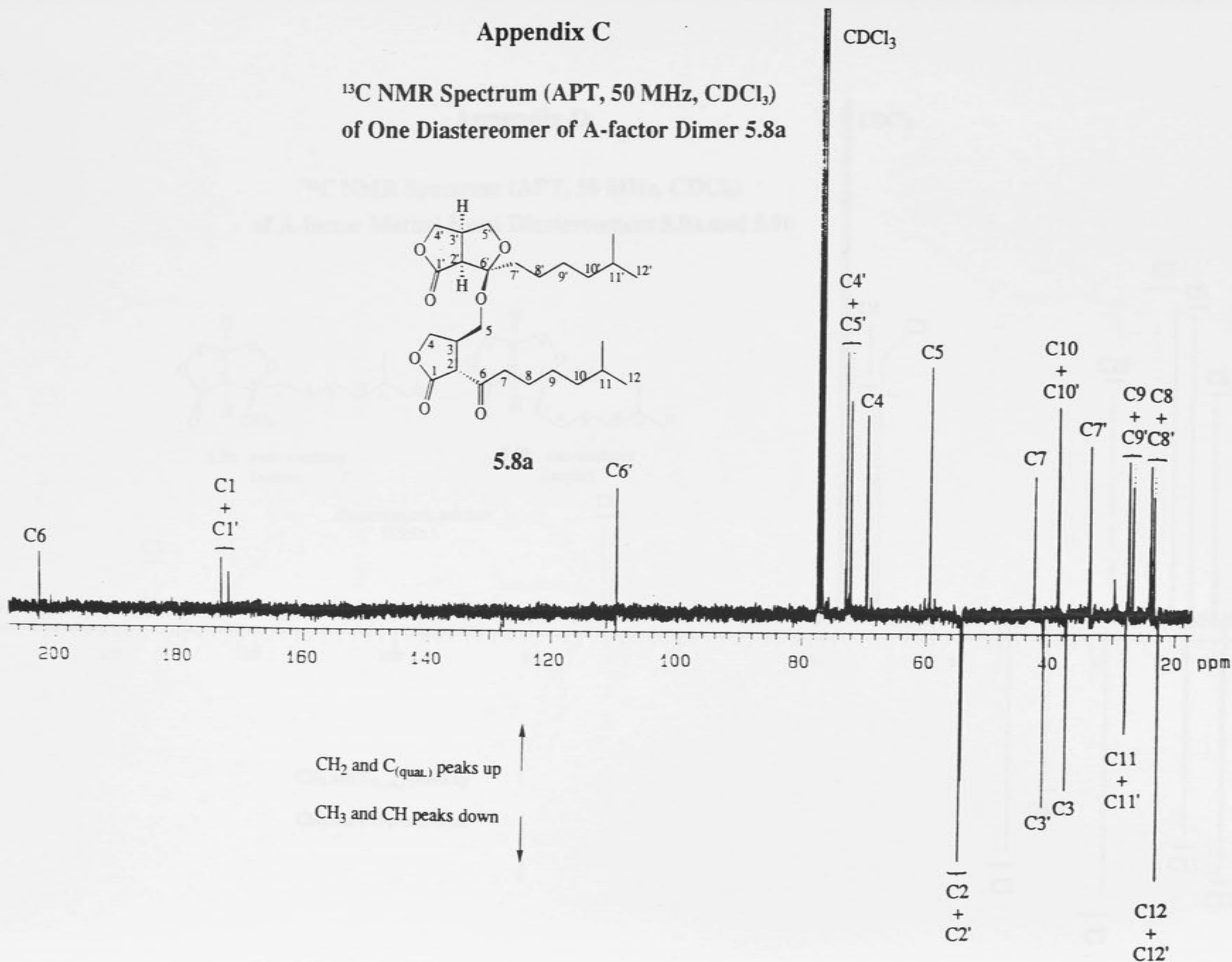


# Appendix C

$^{13}\text{C}$  NMR Spectrum (APT, 50 MHz,  $\text{CDCl}_3$ )  
of One Diastereomer of A-factor Dimer 5.8a

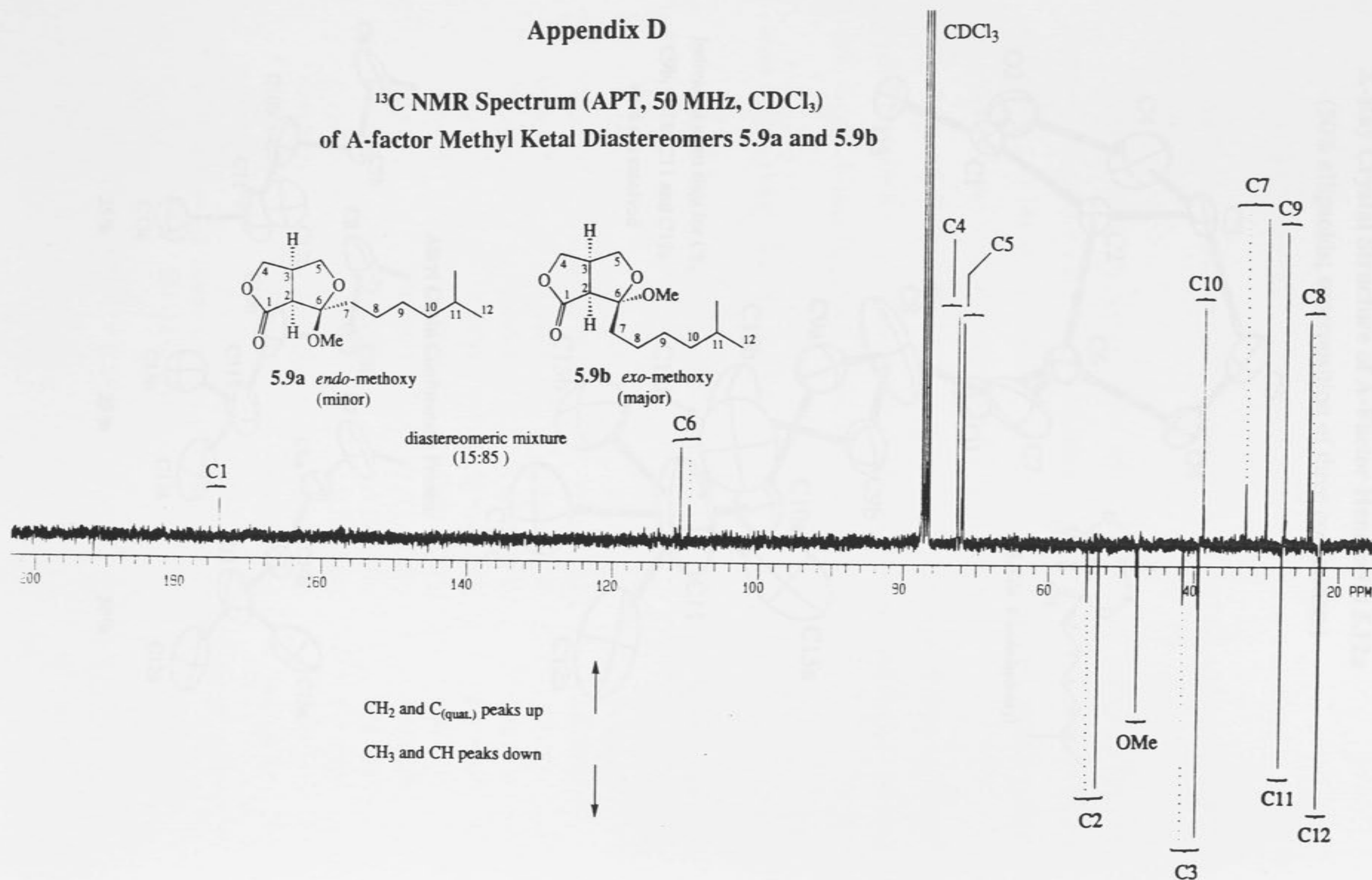


5.8a



# Appendix D

$^{13}\text{C}$  NMR Spectrum (APT, 50 MHz,  $\text{CDCl}_3$ )  
of A-factor Methyl Ketal Diastereomers 5.9a and 5.9b

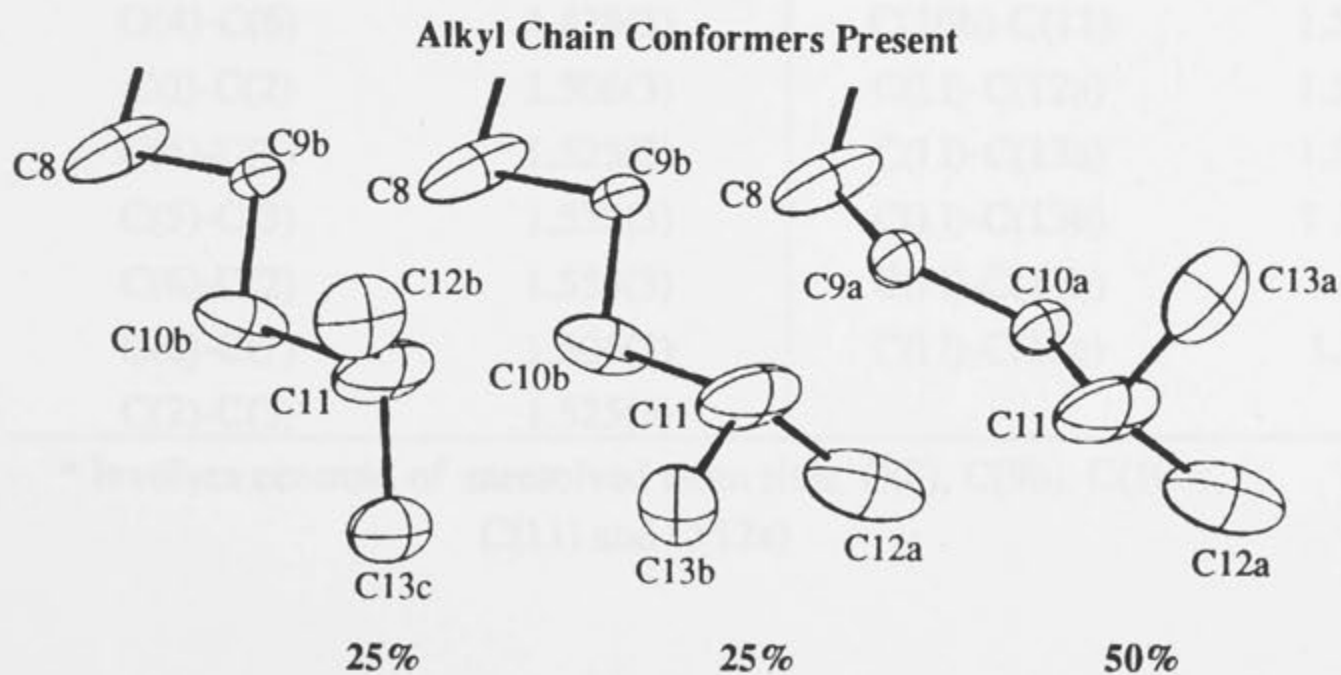
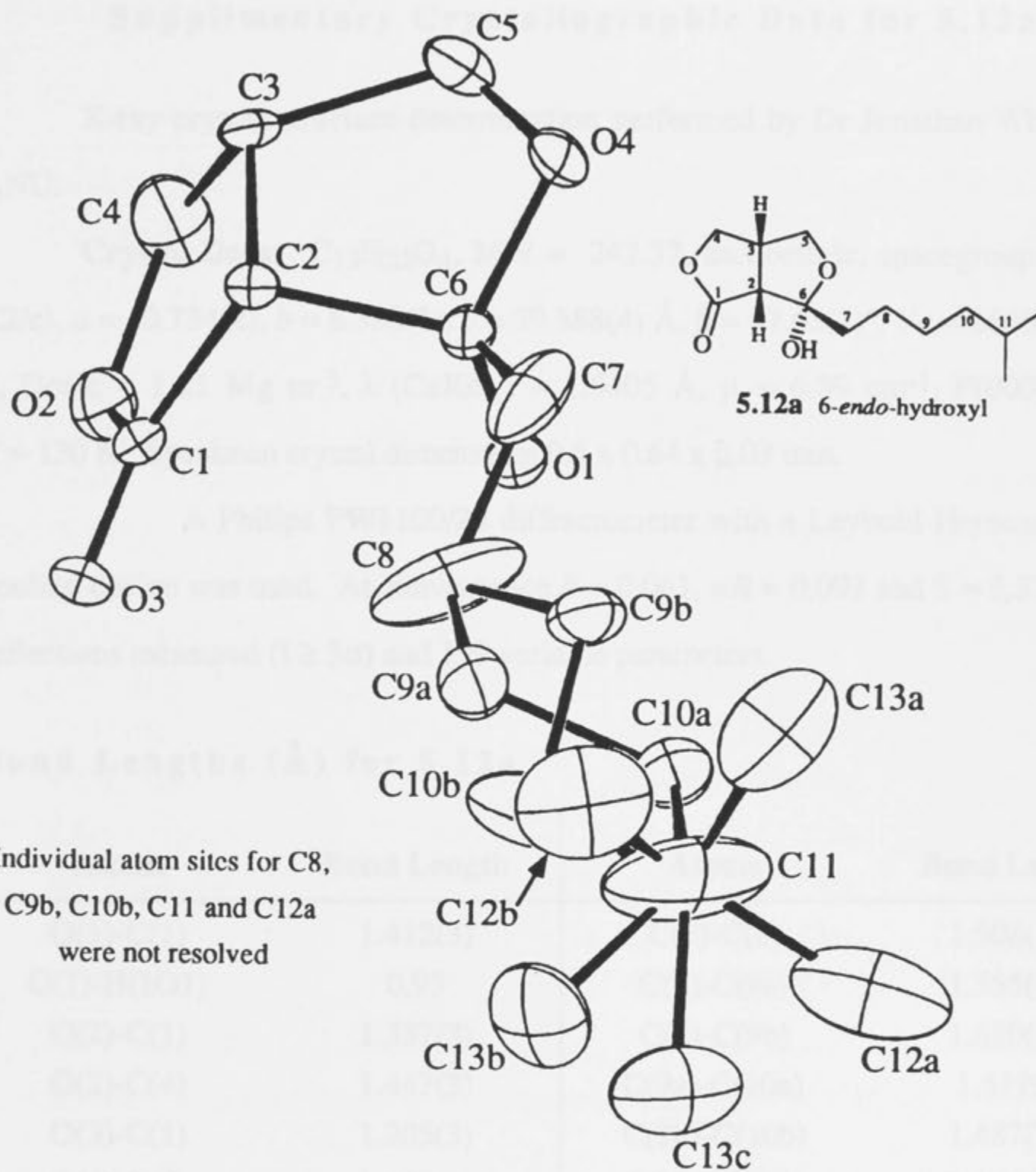




# Appendix Ei

## X-Ray Crystal Structure of A-Factor Hemiketal 5.12a

(50% ellipsoids; superposition of three conformers)



## Appendix Eii

## Supplimentary Crystallographic Data for 5.12a

X-ray crystal structure determination performed by Dr Jonathan White, RSC, ANU.

**Crystal Data:**  $C_{13}H_{22}O_4$ , MW = 242.32, monoclinic, spacegroup I2/a (Alt. C2/c),  $a = 10.734(2)$ ,  $b = 6.330(1)$ ,  $c = 39.588(4)$  Å,  $\beta = 97.63(2)^\circ$ ,  $V_c = 2666.0$  Å<sup>3</sup>,  $Z = 8$ ,  $D_{calc} = 1.21$  Mg m<sup>-3</sup>,  $\lambda$  (CuK $\alpha_1$ ) = 1.5405 Å,  $\mu = 6.39$  cm<sup>-1</sup>,  $F(000) = 1052$ ,  $T = 130$  K. Specimen crystal dimensions 0.5 x 0.64 x 0.03 mm.

A Philips PW1100/20 diffractometer with a Leybold-Heraeus nitrogen cooling device was used. At convergence  $R = 0.061$ ,  $wR = 0.093$  and  $S = 3.57$  for 2012 reflections measured ( $I \geq 3\sigma$ ) and 199 variable parameters.

## Bond Lengths (Å) for 5.12a

Atoms	Bond Length	Atoms	Bond Length
O(1)-C(1)	1.412(3)	C(7)-C(8)	1.508(4)*
O(1)-H(1O1)	0.95	C(8)-C(9a)	1.555(6)*
O(2)-C(1)	1.337(3)	C(8)-C(9b)	1.610(6)*
O(2)-C(4)	1.447(3)	C(9a)-C(10a)	1.519(7)
O(3)-C(1)	1.205(3)	C(9b)-C(10b)	1.487(7)*
O(4)-C(5)	1.432(3)	C(10a)-C(11)	1.655(6)*
O(4)-C(6)	1.428(3)	C(10b)-C(11)	1.562(7)*
C(1)-C(2)	1.506(3)	C(11)-C(12a)	1.573(8)*
C(4)-C(3)	1.523(3)	C(11)-C(13a)	1.507(8)*
C(5)-C(3)	1.532(3)	C(11)-C(13b)	1.73(1)*
C(6)-C(2)	1.554(3)	C(11)-C(12b)	1.67(1)*
C(6)-C(7)	1.506(3)	C(11)-C(13c)	1.56(1)*
C(2)-C(3)	1.525(3)		

\* Involves centroid of unresolved atom sites, C(8), C(9b), C(10b), C(11) and C(12a)



Appendix Eii (continued)

Interbond Angles (°) for 5.12a

Atoms	Angle	Atoms	Angle
C(6)-O(1)-H(1O1)	107	C(5)-C(3)-C(2)	104.5(2)
C(1)-O(2)-C(4)	111.5(2)	C(6)-C(7)-C(8)	115.0(2)*
C(5)-O(4)-C(6)	108.0(2)	C(7)-C(8)-C(9a)	128.7(3)*
O(2)-C(1)-O(3)	121.1(2)	C(7)-C(8)-C(9b)	98.5(3)*
C(2)-C(1)-O(3)	128.1(2)	C(8)-C(9a)-C(10a)	106.4(4)*
O(2)-C(4)-C(3)	107.3(2)	C(8)-C(9b)-C(10b)	106.3(4)*
O(4)-C(5)-C(3)	106.3(2)	C(9a)-C(10a)-C(11)	104.2(4)*
O(1)-C(6)-O(4)	110.4(2)	C(9b)-C(10b)-C(11)	110.9(5)*
O(1)-C(6)-C(2)	106.3(2)	C(10a)-C(11)-C(12a)	93.0(3)*
O(1)-C(6)-C(7)	112.8(2)	C(10a)-C(11)-C(13a)	104.7(4)*
O(4)-C(6)-C(2)	102.8(2)	C(12a)-C(11)-C(13a)	101.9(4)*
O(4)-C(6)-C(7)	107.1(2)	C(10b)-C(11)-C(12a)	121.5(4)*
C(2)-C(6)-C(7)	116.9(2)	C(10b)-C(11)-C(13b)	96.8(5)*
C(1)-C(2)-C(6)	113.5(2)	C(12a)-C(11)-C(13b)	109.4(5)*
C(1)-C(2)-C(3)	104.8(2)	C(10b)-C(11)-C(12b)	96.8(6)*
C(6)-C(2)-C(3)	104.2(2)	C(10b)-C(11)-C(13c)	114.5(6)*
C(4)-C(3)-C(5)	114.4(2)	C(12b)-C(11)-C(13c)	97.4(7)*
C(4)-C(3)-C(2)	104.2(2)		

\* Involves centroid of unresolved atom sites, C(8), C(9b), C(10b), C(11) and C(12a)

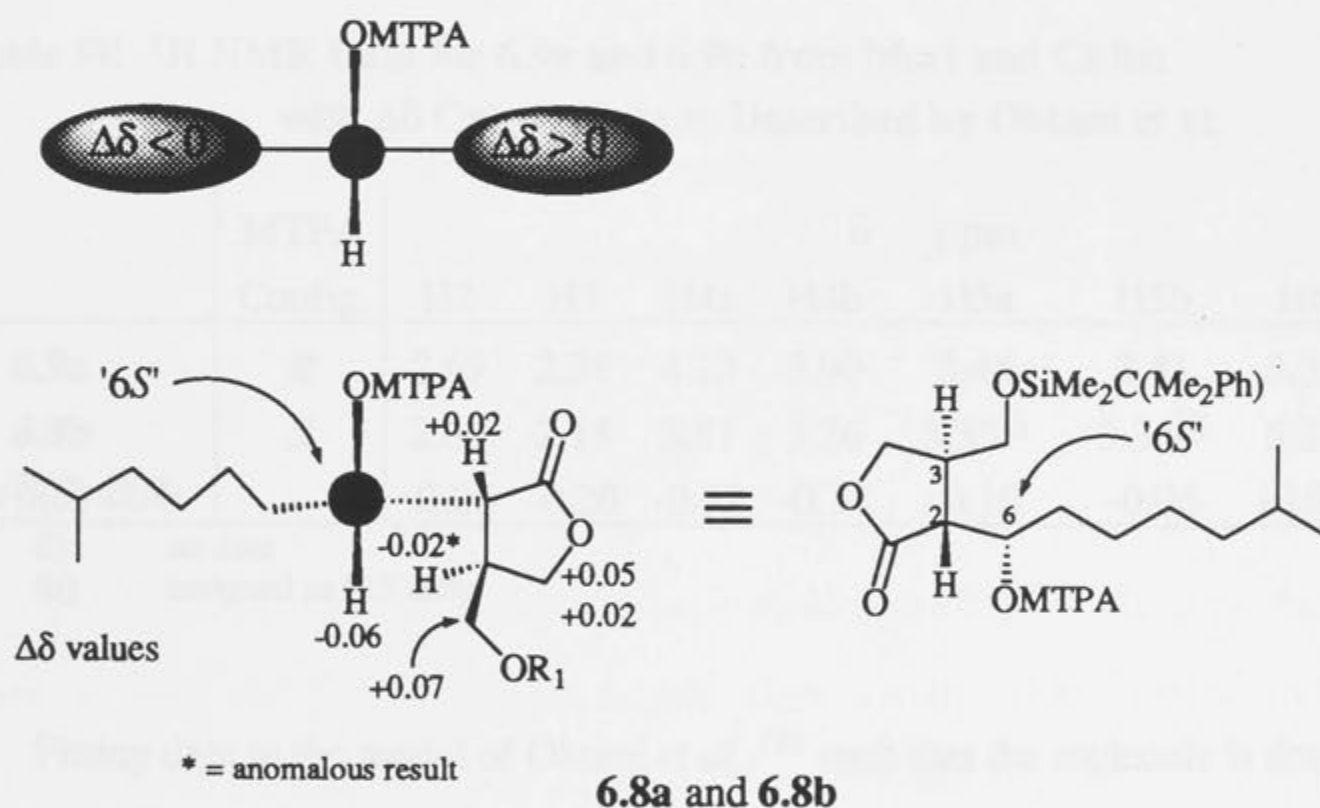




## Appendix F (Continued)

The anomalous sign of the  $\Delta\delta$  value for H3 [calculated above,  $\Delta\delta = \delta(S) - \delta(R)$ , as defined by Ohtani<sup>178</sup>] is a serious defect in the data since Ohtani and coworkers recommend abandonment of an attempted analysis if  $\Delta\delta$  values of mixed signs are obtained for protons on the same side of the 'MTPA plane'.<sup>178</sup> Confusingly, however, in a diagram accompanying these data Mori and Chiba show the  $\Delta\delta$  value for H3 with the same sign as those displayed by nearby resonances.<sup>30</sup>

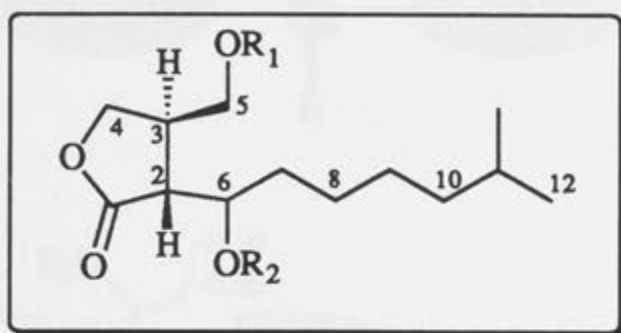
Fitting the  $^1\text{H}$  NMR information (ignoring the  $\Delta\delta$  value for H3) to the model of Ohtani *et al.*,<sup>178</sup> such that the molecule is drawn with the protons having positive  $\Delta\delta$  values on the right hand side and those with a negative  $\Delta\delta$  on the left, gives:



Thus, other than for H3, all  $\Delta\delta$  data measured indicate the assignment of '6S' configuration to butanolide derivatives 6.8a and 6.8b, and hence to the parent butanolide 1.5. Mori and Chiba, however, using the same data have erroneously assigned '6R' configuration.<sup>30</sup>

Appendix F (Continued)

ii) Butanolide 1.2 (factor-I)



1.2  $R_1, R_2 = H$

6.9a  $R_1 = -SiMe_2(CMe_2Ph)$   
 $R_2 = (R)$ -MTPA

6.9b  $R_1 = -SiMe_2(CMe_2Ph)$   
 $R_2 = (S)$ -MTPA

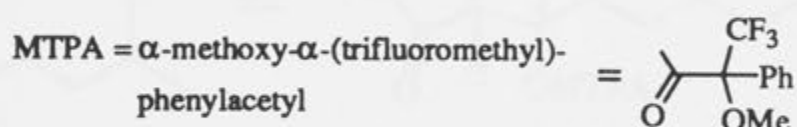


Table Fii  $^1H$  NMR Data for 6.9a and 6.9b from Mori and Chiba  
 with  $\Delta\delta$  Calculations as Described by Ohtani et al.

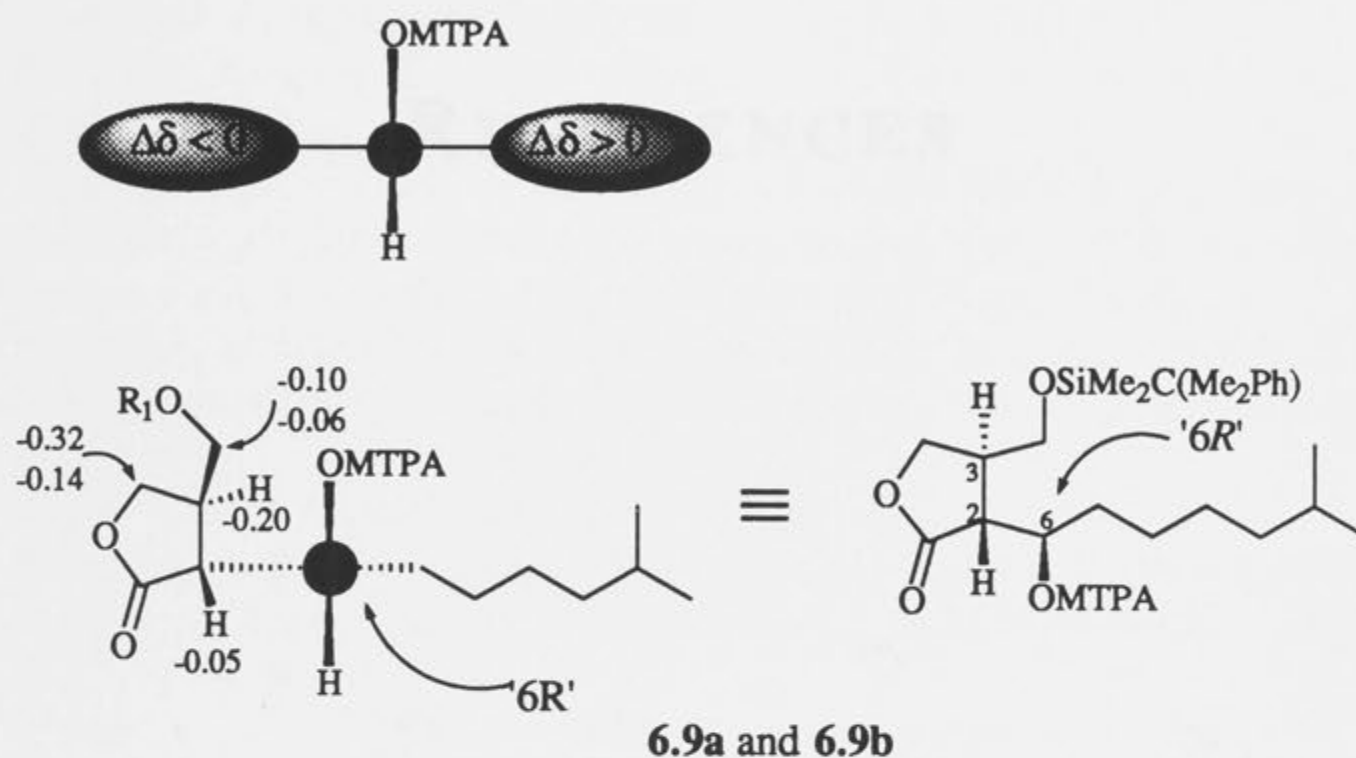
	MTPA Config.	$\delta$ ppm							
		H2	H3	H4a	H4b	H5a	H5b	H6	H7-12
6.9a	<i>R</i>	2.69	2.38	4.13	3.90	3.45	3.41	5.31	ii
6.9b	<i>S</i>	2.64	2.18	3.81	3.76	3.35 <sup>iii</sup>	3.35 <sup>iii</sup>	5.23	ii
$\Delta\delta = \delta(S) - \delta(R)$		-0.05	-0.20	-0.32	-0.14	-0.10	-0.06	-0.08	-

ii) no data  
 iii) assigned as 'H5' only

Fitting data to the model of Ohtani *et al.*,<sup>178</sup> such that the molecule is drawn with the protons having positive  $\Delta\delta$  values [calculated above,  $\Delta\delta = \delta(S) - \delta(R)$ ] on the right hand side and those with a negative  $\Delta\delta$  on the left, gives:



## Appendix F (Continued)



Thus all  $\Delta\delta$  values support the assignment of '6R' configuration to the butanolide derivatives **6.9a** and **6.9b**, and hence to the parent butanolide autoregulator **1.2**. Again however, Mori and Chiba derive the erroneous assignment '6S', by analysis of the same data.

### Conclusions

The  $^1\text{H}$  NMR data for the MTPA ester derivatives **6.8a**, **6.8b** and **6.9a**, **6.9b** as reported by Mori and Chiba<sup>30</sup> *do not* support their stereochemical assignments of the parent butanolide autoregulators **1.5** and **1.2**. Careful re-analysis of their data indicates the opposite C6 stereochemical conclusions for both **1.5** and **1.2**. The reported erroneous assignments may reflect the confusing definition of  $\Delta\delta$  employed in their analysis of the data.<sup>30</sup> Note also however, that the lack of complementary  $\Delta\delta$  values for the protons of the alkyl chains (H7-H12) makes any assignment based on these data unreliable.<sup>178</sup> Also of concern is that the absolute  $\Delta\delta$  values do not show the expected reduction in magnitude with increasing distance from the MTPA moiety,<sup>178</sup> although this may reflect conformational effects.

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